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AUTHOR'S ERRATA AND EMENDATIONS

Page 48, table 3, and page 54, table 7, unit over last column, "Liters÷grams" should be "Milliliters÷grams."

Page 90, table 3, center heading "100 EARLY SINGLE CROSSES CONTINUED" should be "PRIDE OF SALINE SINGLE CROSSES."

Page 139, line 5, "1.23" should be "1.22," line 8, "81.3" should be "82.0;" line 8, "(1÷1.23)" should be "(1÷1.22);" line 16, "74.7" should be "74.6."

Pages 153 and 155, illustrations appearing as figure 2 and figure 4 should be reversed; legends are in correct order.

Page 200, in box headings for table 5, >55 should be <55, and <70 should be >70.

Page 215, line 3, "(9)³" should be "(11)³."

Page 216, line 16, "(5)" should be "(7);" line 17, "(6)" should be "(8);" line 18, "(8)" should be "(10)."

Page 217, seventeenth line from bottom, "(9)" should be "(11)."

Page 219, last line, "(7)" should be "(9)."

Page 238, line 17, "0.307876" should be "0.307877," line 20, "=" should be "= −;" and "0.00821822" should be "0.000821822;" line 23, "93" should be "92;" twelfth line from bottom, "0.96047" should be "0.967047," seventh line from bottom, last digit, "6" should be "2;" second line from bottom, last digit, "7" should be "6."

Page 239, line 21, small 4 refers to footnote.

Page 239, line 24, last digit, "6" should be "8;" line 28, last digit, "6" should be "8."

Page 240, reference 9 should read "Yule, G. U., and Kendall, M. G.," and "Ed. 10" should be "Ed. 11, 1937."

Page 400, line 6, "insignificant" should be "significant."

Page 403, table 1, column 7, last line, "1.00" should be "1.09."

Page 545, table 2, heading of last column should be "<200."

Page 564, table 1, footnote 3, the terms enclosed in brackets, "n_c" should be "$n\sqrt{c}$" and "n_k" should be "$n\sqrt{k}$."

Page 665, line 5, insert "on the parts" between the words "treatments" and "of."

Page 751, table 4, between lines 9 and 10, insert "4" and "$RrF_1F_1F_2F_2D_1D_1D_2d_2$."

Page 796, plate 1 D, "42 times natural size" should be "740 times natural size."

Page 822, line 7, "the varied" should be "balls varied."

Page 873, line 11, insert "p. 867" in the parenthesis.

JOURNAL OF AGRICULTURAL RESEARCH

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INVERSION OF SUCROSE IN THE DIFFERENT PARTS OF THE SUGARCANE STALK [1]

By J. I. LAURITZEN, *senior physiologist, Division of Sugar Plant Investigations, Bureau of Plant Industry*, and R. T. BALCH, *senior chemist, Carbohydrate Research Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture* [2]

INTRODUCTION

As sugarcane (*Saccharum* spp.) matures, the sucrose content tends to become more or less equal throughout the millable portion of the stalk. In Louisiana, as in many Temperate Zone and some Torrid Zone sugar-producing countries, sugarcane never reaches maturity. If the stalk, as it is prepared for the mill,[3] is sectioned into three equal lengths and each section analyzed separately, it will be found that there is a considerable gradient in sucrose content from the top to the middle to the bottom section, the smallest amount being in the top third and the largest in the bottom third. As a rule, the difference is greater between the top and middle thirds than between the middle and bottom thirds. This gradient varies with the variety and the maturity of the cane. There also is an obvious difference in the vegetative condition of the tissues in these three parts of the cane stalk, particularly between the top and the bottom thirds, the middle third resembling more closely the bottom section. The top third is greener, softer, and more active physiologically than the other two sections.

The object of the investigation reported herein was to determine what bearing, if any, this difference in sucrose content and vegetative condition has on inversion of sucrose in harvested sugarcane.

REVIEW OF LITERATURE

Coates (*3*) reported more inversion of sucrose in the top third than in the bottom third of the stalk of certain varieties of sugarcane (P. O. J. 36 and P. O. J. 234) when whole stalks were stored in the shade and sectioned and analyzed after different periods of time. Cross and Harris (*4*), working in Argentina, indicated that in the varieties P. O. J. 36, P. O. J. 213, P. O. J. 234, P. O. J. 139, Kavangire, and Criolla the upper half of the stalk had a smaller sucrose content than the lower half. When samples of the upper and lower halves of 10 stalks of these varieties were stored for 10 days under comparable conditions, the percentage of inversion was greater in the upper half than in the lower half in all varieties except Criolla. In the case of Criolla the

[1] Received for publication April 19, 1940.

[2] For some of the Brix analyses and the apparent sucrose analyses the writers are indebted to R. B. Bisland and D. D. Sullivant, both formerly of the Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] *In the preparation of sugarcane for the mill the stalk is cut off at the ground level, stripped of its leaves,* and topped at a point as near the terminal bud as is justifiable from the standpoint of sucrose content. Change in color and appearance of the stalk has been used as an indication of this point. This point, or more appropriately region, moves up the stalk as the cane matures. Recently the point of topping has been influenced by the manner of maturing in different varieties (*2*).[4] In connection with the experimental work reported in this paper, the point of cutting was made as uniform as practicable.

[4] Italic numbers in parentheses refer to Literature Cited, p. 16.

inversion was greater in the lower half of the stalk than in the upper half in two out of three experiments. In all varieties the weight loss during storage was greater in the upper half of the stalk than in the lower half. Cross and Harris state that inversion was due to invertase present in the cane and that the concentration of invertase was relatively higher in the upper part of the stalk than in the lower part.

MATERIAL AND METHODS

Five experiments were conducted at Houma, La., during 4 years, 1932, 1933, 1934, and 1936, in which whole-stalk samples and one-third-stalk samples from the same lots of cane were placed in storage. In the first two experiments the samples were stored under controlled conditions of temperature and humidity, and in the last three experiments they were stored in the shade in an open shed. In the first two experiments sectioned and whole-stalk samples were stored in separate piles on a rack, the stalks lying parallel with all the cut ends exposed to the air; in the last three the samples were stored one layer thick on a rack, one whole-stalk sample alternating with the sections of another sample, the ends of the sections being separated by 4 to 6 inches. This latter arrangement was adopted in order to insure a more uniform evaporation from all samples than obtained in samples stored under controlled storage conditions.

Shade was provided by means of a shed 20 feet wide, 50 feet long, and 8 feet high, facing north, open in the front, and with provision for opening the lower 4 feet of the back.

Constant temperature was maintained in insulated rooms 10 feet wide, 12 feet long, and 9.5 feet high by means of refrigeration and electrical heating units thermoelectrically controlled. Humidity control was obtained by using water humidifiers and calcium chloride dryers.

The variety P. O. J. 36–M was used in four experiments and Co. 290 in one.

In a given experiment a quantity of sugarcane from a plot of uniform stand was harvested and thoroughly mixed as it was placed in a long pile, the stalks lying parallel with the butts in one direction. Samples consisting of 30 stalks each were then selected by drawing stalks at random from the pile. Both ends of each stalk were trimmed so that the cuts were at right angles to the length of the stalk. These 30-stalk samples were divided into two lots. The stalks of one lot were cut into three equal lengths (each whole stalk forming three short lengths), while the other lot was held as whole-stalk samples. Check samples were analyzed immediately, and the remaining sectioned and whole-stalk samples were placed in storage. After different periods of storage whole-stalk samples were cut into three equal lengths and analyzed along with samples sectioned before storage. In the last experiment conducted (with Co. 290), whole-stalk samples were analyzed at the various periods of storage. Five 30-stalk samples divided into sections, as well as five of the whole-stalk samples, were analyzed for each period of storage, and the results in each case are averages of five separate analyses.

The methods of analysis were the same as those employed in earlier work (*6*).

The sugar yields were calculated by means of the Winter-Carp-Geerligs formula (*5*), the simplified method developed by Arceneaux (*1*) being used. The yields were corrected for loss in weight of stored cane. Varietal milling factors were not used in connection with any of the calculated yields.

In certain instances, the yields of sucrose per ton of cane for the whole-stalk sample were calculated from data obtained from the sectioned sample. The yields were first calculated for the individual sectioned sample, corrected for weight loss in case of stored samples, and multiplied by a ratio factor obtained by dividing the original weight of the sectioned sample by the original weight of the whole-stalk sample. The yields thus obtained from the three sections of a particular sample were added to obtain the yield for the whole-stalk sample. In each of the experiments the ratio factors were obtained from the sectioned check samples and the samples sectioned before storage. The ratios of the top-, middle-, and bottom-section weights to the weight of the whole-stalk sample, expressed as percentages, were very constant in the samples of a given experiment and fairly constant from experiment to experiment (table 1).

TABLE 1.—*Average weights of whole-stalk samples, the ratio factors of sectioned samples, and the standard errors of these factors*

[The weights and ratios are the averages of the sectioned check samples and those sectioned before storage in each of 5 experiments at the time they were initiated]

Variety	Date experiment started	Samples used in calculations	Average weight of whole-stalk samples	Ratio of weight of sections to weight of whole stalks		
				Top	Middle	Bottom
	1932	*Number*	*Pounds*	*Percent*	*Percent*	*Percent*
P. O. J. 36-M	Dec. 8	25	46.53	28.97±.28	33.57±.08	37.46±.3
	1933					
Do	Nov. 8	40	48.94	28.86±.23	33.35±.12	37.79±.2
	1934					
Do	Nov. 2	25	48.19	28.83±.23	34.39±.09	36.76±.1
Do	Nov. 28	15	52.90	30.59±.51	33.44±.40	35.97±.1
	1936					
Co. 290	Oct. 30	20	71.70	29.70±.15	33.18±.09	37.13±.1

These same factors were used to calculate the probable original weights of sectioned samples of cane sectioned after storage. The initial weight of the whole-stalk sample and the weights of the sample sectioned after storage being known, it was possible to calculate the percentage loss of weight in the top-, middle-, and bottom-third sections of the whole-stalk sample.

The inverse correlation between the percentage of increase in Brix and the percentage of loss in weight during storage was calculated for the top and bottom portions of cane sectioned before storage, the data obtained from all five experiments being used. The correlation coefficient and standard error for the top sections were 0.949 and 0.033 and for the bottom sections 0.896 and 0.063, respectively. The percentage of increase in Brix, of course, is not equivalent to the percentage of loss in weight, but it definitely indicates the trends in loss in weight.

In certain instances the percentage of loss of sucrose in juice was calculated. In obtaining these values the apparent sucrose in juice from stored samples was corrected for weight loss before the percentage of loss was determined.

The statistical calculations given in tables 2 and 3 are based on the analysis of variance. The values given represent the difference in purity required for significance when $P=0.05$ and when $P=0.01$; in other words, the differences given may be expected to occur by chance alone 5 times in 100 when $P=0.05$ and 1 time in 100 when $P=0.01$.

EXPERIMENTAL DATA

Results obtained in connection with other experiments show that the loss in weight is primarily due to the loss of moisture and that the loss of moisture from cane of a given variety is a function of the saturation deficit of the air, independent of temperature, at least over a range of 45° to 65° F. (7.2° to 18.3° C.). In earlier work (*6*, *7*) it was shown that loss of moisture is an important factor in bringing about inversion of sucrose in sugarcane, and, conversely, the inhibition of moisture loss by sprinkling or by storage at high humidity checks inversion. These results are confirmed by data obtained in connection with two of the experiments reported in this paper. In one experiment the samples were stored at relative humidities of 96 and 72 percent at 65° F. and in the other at relative humidities of 98 and 71 percent at 66°. The high humidity materially checked inversion in both experiments. Because no other significant conclusions can be drawn from the data obtained from samples stored at high humidities, these data will not be presented.

The data obtained from cane stored at temperatures of 65° and 66° F. and relative humidities of 72 and 71 percent, respectively, and under dry conditions in the shade (tables 2, 3, and 4, and figs. 1 and 2) reveal some interesting relationships.

Because of the profound effect drying out of cane has on inversion of sucrose in some varieties (P. O. J. 36–M being one) and the fact that there is considerable loss of moisture through the cut ends of the stalks (*8*), it was expected that there would be more loss of moisture and hence greater inversion of sucrose in cane stored as sections. The total percentage of loss in weight of the three sections was greater than that of the whole-stalk samples in three of the four experiments (Nos. 3, 4, and 5, but not in No. 2, table 4), in which the time factor was comparable. In the experiment in which the exception occurred (No. 2), the samples were piled upon each other, thus preventing uniform exposure, whereas in the other three experiments the exposure of all samples was similar. It was concluded that the manner in which the samples were stored may have been responsible for this difference in weight loss in the different experiments. That this conclusion is justified is indicated by the greater variability in loss of moisture and inversion of sucrose in experiment 2 than in the other three experiments. The amount of inversion in an individual sample of experiment 2 was definitely associated with the amount of moisture loss.

The combined loss of sucrose from the three sections of cane sectioned either before or after storage was correlated in the main with the total loss of moisture (table 5).

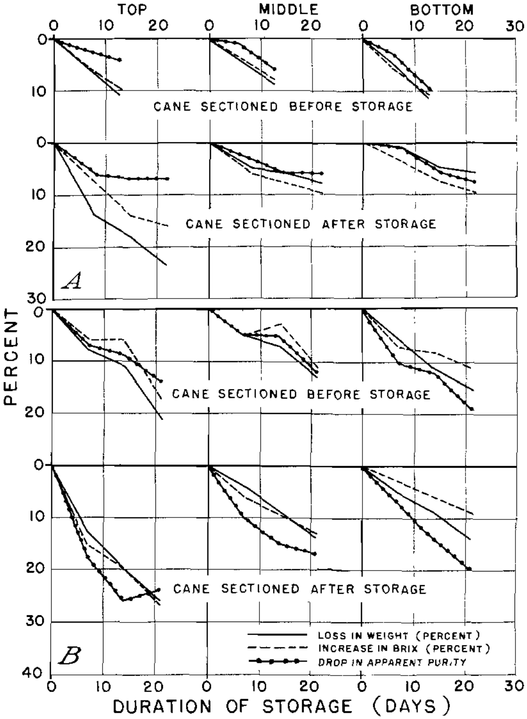


FIGURE 1.—Loss in weight, increase in Brix, and drop in apparent purity in the top-, middle-, and bottom-third portions of the sugarcane stalk sectioned before and after storage: *A*, P. O. J. 36–M, plant cane, stored from December 8 to 21 and December 30, 1932, at a temperature of 65° F. and a relative humidity of 72 percent; *B*, P. O. J. 36–M, first-year stubble, stored from November 8 to 29, 1933, at a temperature of 66° and a relative humidity of 71 percent. Analyses were made at different intervals during storage.

... portions of the sugarcane stalk (P. O. J. 36–M) sectioned before storage and after storage under conditions of controlled temperature and relative humidity

...f sectioning cane	Section of cane	Conditions of storage: Temperature	Conditions of storage: Relative humidity	Date of analysis	Duration of storage	Brix	Apparent sucrose	Apparent purity	Drop in apparent purity	Yield of available sucrose per ton of cane [1]	Juice extraction	Acidity (0.1 N NaOH per 10 cc. of juice)	pH	Dry substance [1]	Sucrose [1 2]	Invert sugars [1 2]	Total sugars [1 2]	Ash [1 2]	Organic nonsugars [1 2]
		°F.	Pct.	1932	Days	°	Pct.			Lb.	Pct.	Cc.		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
...ane:																			
...fore storage	Top third	65	72	Dec. 8	0	14.14	10.13	71.6	----	129.6	64	2.40	5.18	13.31	77.08	13.24	90.32	4.29	5.39
				Dec. 14	6	14.85	10.37	69.8	1.8	123.9	60	2.45	5.30	13.46	76.18	13.96	90.14	4.17	5.69
				Dec. 21	13	15.57	10.51	67.5	4.1	114.8	60	2.50	5.31	13.37	75.20	14.46	89.66	3.99	6.35
	Middle third	65	72	Dec. 8	0	16.09	13.54	84.2	----	190.8	62	1.75	5.33	15.62	87.48	6.08	93.56	2.64	3.80
				Dec. 14	6	16.77	13.94	83.1	1.1	188.3	60	1.80	5.38	15.76	86.59	6.43	93.02	2.66	4.32
				Dec. 21	13	17.43	13.71	78.7	5.5	169.9	62	2.00	5.34	15.58	83.31	9.31	92.62	2.74	4.04
	Bottom third	65	72	Dec. 8	0	16.97	15.27	90.0	----	222.5	61	1.45	5.41	16.65	91.95	2.65	94.60	1.96	3.44
				Dec. 14	6	17.97	15.68	87.3	2.7	214.2	57	1.50	5.45	16.85	89.69	4.87	94.56	1.81	3.63
				Dec. 21	13	18.75	14.99	79.9	10.1	181.7	55	1.75	5.34	16.46	83.28	10.11	93.39	1.96	4.65
...er storage	Top third	65	72	Dec. 8	0	14.14	10.13	71.6	----	129.6	64	2.40	5.18	13.31	77.08	13.24	90.32	4.29	5.39
				Dec. 16	8	15.27	10.10	66.1	5.5	104.3	56								
				Dec. 23	15	16.09	10.42	64.8	6.8	101.4	58	2.70	5.20	12.43	72.36	18.23	90.50	4.19	5.22
				Dec. 30	22	16.46	10.68	64.9	6.7	96.0	52	2.75	5.25	12.03	72.66	17.40	90.06	4.14	5.80
	Middle third	65	72	Dec. 8	0	16.09	13.54	84.2	----	190.8	62	1.75	5.33	15.62	87.48	6.08	93.56	2.64	3.80
				Dec. 16	8	16.99	13.76	81.0	3.2	180.6	59								
				Dec. 23	15	17.38	13.59	78.2	6.0	173.5	61	2.05	5.30	16.03	83.52	9.47	92.99	2.66	4.35
				Dec. 30	22	17.65	13.73	77.8	6.4	170.1	60	2.05	5.29	15.77	83.45	9.92	93.37	2.59	4.04
	Bottom third	65	72	Dec. 8	0	16.97	15.27	90.0	----	222.5	61	1.45	5.41	16.65	91.95	2.65	94.60	1.96	3.44
				Dec. 16	8	17.59	15.70	89.3	.7	225.5	59								
				Dec. 23	15	18.29	15.42	84.3	5.7	207.5	60	1.65	5.43	17.11	87.74	6.15	93.89	2.00	4.11
				Dec. 30	22	18.62	15.33	82.3	7.7	200.7	53	1.65	5.35	17.15	87.17	8.06	95.23	1.77	3.00
...ear stubble:				1933															
...ore storage	Top third	66	71	Nov. 8	0	12.62	7.85	62.2	----	90.1	55	1.94	5.14	11.85	69.28	19.76	89.04	5.36	5.60
				Nov. 15	7	13.43	7.38	55.0	7.2	69.3	55								
				Nov. 22	14	13.42	7.16	53.4	8.8	62.2	55								
				Nov. 29	21	14.78	7.20	48.7	13.5	49.5	52	2.01	5.22	11.00	58.08	29.68	87.76	5.53	6.71
	Middle third	66	71	Nov. 8	0	15.16	11.88	78.4	----	160.9	59	1.51	5.30	14.65	83.28	9.27	92.55	3.46	3.99
				Nov. 15	7	15.90	11.63	73.1	5.3	143.2	59								
				Nov. 22	14	15.67	11.48	73.3	5.1	138.8	58								
				Nov. 29	21	16.78	11.14	66.4	12.0	117.1	58	2.01	5.06	14.06	73.40	17.26	90.66	3.64	5.70
	Bottom third	66	71	Nov. 8	0	16.38	14.31	87.4	----	205.5	58	1.07	5.41	16.02	89.89	4.05	93.94	2.24	3.82
				Nov. 15	7	17.53	13.59	77.5	9.9	171.2	56								
				Nov. 22	14	17.75	13.42	75.6	11.8	159.1	57								
				Nov. 29	21	18.24	12.42	68.1	19.3	130.5	56	1.79	4.83	15.16	74.86	17.82	92.08	2.37	4.95

After storage	Top third	66	71	Nov. 8	0	12.62	7.85	62.2		90.1	55	1.94	5.14	11.85	69.28	19.76	89.04	5.36	5.60
				Nov. 15	7	14.51	6.41	44.2	18.0	41.2	53								
				Nov. 22	14	15.12	5.42	35.8	26.4	17.7	53								
				Nov. 29	21	16.06	6.22	38.7	23.5	25.0	51	2.20	5.22	11.46	50.71	37.18	87.89	5.40	6.71
	Middle third	66	71	Nov. 8	0	15.16	11.88	78.4		160.9	59	1.51	5.30	14.65	83.28	9.27	92.55	3.46	3.99
				Nov. 15	7	16.13	11.05	68.5	9.9	131.9	58								
				Nov. 22	14	16.53	10.44	63.2	15.2	111.1	60								
				Nov. 29	21	17.13	10.48	61.2	17.2	102.1	57	1.74	5.27	14.35	69.25	22.03	91.28	3.57	5.15
	Bottom third	66	71	Nov. 8	0	16.38	14.31	87.4		205.5	58	1.07	5.41	16.02	89.89	4.05	93.94	2.24	3.82
				Nov. 15	7	16.84	13.48	80.0	7.4	176.1	56								
				Nov. 22	14	17.36	12.67	73.0	14.4	148.8	57								
				Nov. 29	21	17.78	12.00	67.5	19.9	127.2	57	1.45	5.15	15.03	74.84	18.37	93.21	2.25	4.54
Difference[3] for $P=0.05$									4.07										
Difference[3] for $P=0.01$									5.38										

[1] Corrected for weight loss.
[2] Percentage based on dry substance.
[3] The value given is based on cane stored at a temperature of 66° F. and a relative humidity of 71 percent.

storage and after storage in the shade for different periods

P. O. J. 36-M, SECOND-YEAR STUBBLE

Section of cane	Date of analysis	Duration of storage	Cane sectioned before storage: Brix	Apparent sucrose	Apparent purity	Drop in apparent purity	Yield of available sucrose per ton of cane [1]	Juice extraction	Cane sectioned after storage: Brix	Apparent sucrose	Apparent purity	Drop in apparent purity	Yield of available sucrose per ton of cane [1]	Juice extraction
	1934	*Days*	°	*Percent*			*Pounds*	*Percent*	°	*Percent*			*Pounds*	*Percent*
	Nov. 2	0	13.31	8.77	65.9		105.5	58	13.31	8.77	65.9		105.5	58
third	Nov. 9	7	14.39	5.60	38.9	27.0	27.3	59	15.47	5.40	34.9	31.0	17.3	58
	Nov. 16	14	15.20	5.01	33.0	32.9	10.6	60	16.62	4.40	26.5	39.4	(2)	58
	Nov. 2	0	15.87	12.89	81.2		178.2	59	15.87	12.89	81.2		178.2	59
ddle third	Nov. 9	7	16.75	9.51	56.8	24.4	93.1	61	16.93	8.97	53.0	28.2	81.9	61
	Nov. 16	14	17.72	8.49	47.9	33.3	63.0	64	17.38	7.04	40.5	40.7	38.7	66
	Nov. 2	0	17.17	15.14	88.2		218.4	57	17.17	15.14	88.2		218.4	57
tom third	Nov. 9	7	18.33	11.41	62.2	26.0	120.8	60	18.00	12.60	70.0	18.2	148.8	59
	Nov. 16	14	19.40	10.40	53.6	34.6	89.2	57	18.44	10.07	54.6	33.6	93.2	63
ifference for $P=0.05$						1.77						1.77		
ifference for $P=0.01$						2.36						2.30		
third	Nov. 28	0	13.70	8.96	65.4		107.2	61	13.70	8.96	65.4		107.2	61
	Dec. 5	7	14.79	8.52	57.6	7.8	81.9	56	15.19	8.31	54.7	10.7	71.8	57
ddle third	Nov. 28	0	16.40	13.69	83.5		192.1	64	16.40	13.69	83.5		192.1	64
	Dec. 5	7	16.79	13.77	82.0	1.5	182.7	61	17.11	14.00	81.8	1.7	189.1	63
tom third	Nov. 28	0	17.60	15.81	89.8		230.1	62	17.60	15.81	89.8		230.1	62
	Dec. 5	7	18.33	15.88	86.6	3.2	215.1	62	18.00	15.98	88.8	1.0	215.1	59

CO. 290, FIRST-YEAR STUBBLE

Section of cane	Date of analysis	Duration of storage	Cane sectioned before storage: Brix	Apparent sucrose	Apparent purity	Drop in apparent purity	Yield of available sucrose per ton of cane [1]	Juice extraction	Cane sectioned after storage: Brix	Apparent sucrose	Apparent purity	Drop in apparent purity	Yield of available sucrose per ton of cane [1]	Juice extraction
	1936													
	Oct. 30	0	10.86	5.82	53.6		57.4	63	10.86	5.82	53.6		57.4	63
third	Nov. 4	5	11.39	5.09	44.7	8.9	35.4	62	11.44	4.65	40.6	13.0	25.8	62
	Nov. 9	10	11.41	4.06	35.6	18.0	14.3	63	11.83	4.11	34.7	18.9	12.9	62
	Nov. 16	17	12.08	4.55	37.7	15.9	10.4	62	12.91	4.69	36.3	17.3	17.0	60
	Oct. 30	0	13.64	9.71	71.2		123.7	66	13.64	9.71	71.2		123.7	66
ddle third	Nov. 4	5	14.38	9.75	67.8	3.4	113.5	66	13.87	9.37	67.6	3.6	111.2	66
	Nov. 9	10	14.56	8.97	61.6	9.6	94.5	66	13.84	8.44	61.0	10.2	91.1	64
	Nov. 16	17	14.80	8.99	60.7	10.5	90.2	63	14.69	8.58	58.4	12.8	85.4	64

tom third	Oct. 30	0	15.44	12.46	80.7		171.6	63	15.44	12.46	80.7		171.6	63
	Nov. 4	5	16.27	12.69	78.0	2.7	162.8	63	15.63	12.33	78.9	1.8	174.0	65
	Nov. 9	10	16.28	12.08	74.2	6.5	147.3	65	15.54	11.62	74.8	5.9	148.2	63
	Nov. 16	17	16.94	11.81	69.7	11.0	133.0	63	15.95	11.61	72.8	7.9	144.5	64
ifference for P=0.05						1.86						1.86		
ifference for P=0.01						2.46						2.46		

[1] Corrected for weight loss.
[2] No yield because of low purity.

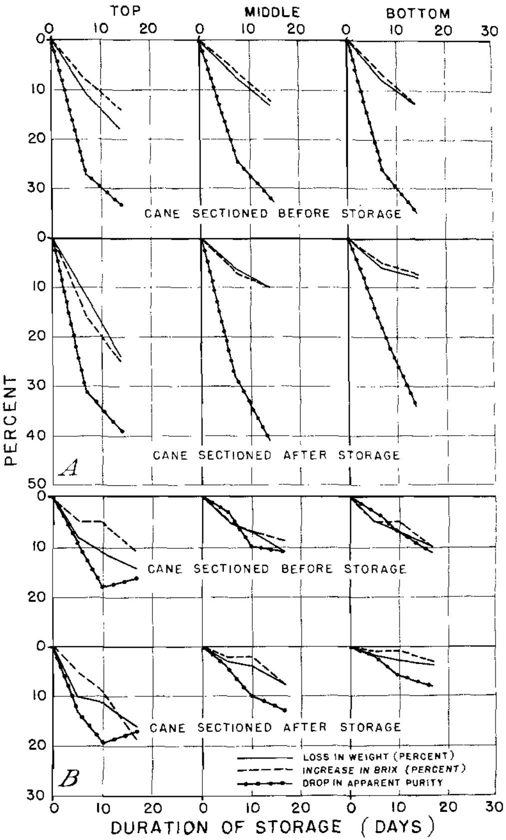
... juice, and loss of available sucrose in the top-, middle-, and bottom-third portions *of the sugarcane stalk sectioned before and after storage*

P. O. J. 36-M

Experiment	Time of sectioning cane	Conditions of storage: Temperature	Conditions of storage: Relative humidity	Conditions of storage: Open shed	Date of analysis	Duration of storage	Weight loss in— Top third	Weight loss in— Middle third	Weight loss in— Bottom third	Weight loss in— Entire stalk	Increase in Brix: Top third	Increase in Brix: Middle third	Increase in Brix: Bottom third	Loss of sucrose in juice: Top third	Loss of sucrose in juice: Middle third	Loss of sucrose in juice: Bottom third	Loss of available sucrose: Top third	Loss of available sucrose: Middle third	Loss of available sucrose: Bottom third
		°F.	Per cent			Days	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
	Plant cane:				*1932*														
1	Before storage	65	72		Dec. 14	6	4.9	3.5	4.8	4.4	5.0	4.2	5.9	2.7	0.7	2.2	4.4	1.3	3.7
		65	72		Dec. 21	13	10.9	8.7	11.5	10.4	10.1	8.3	10.5	7.6	7.5	13.1	11.4	11.0	18.3
	After storage	65	72		Dec. 16	8	14.4	4.9	1.0	6.0	8.0	5.6	3.7	14.6	3.3	+1.8	19.5	5.3	+1.3
		65	72		Dec. 23	15	18.0	5.6	4.6	8.9	13.8	8.0	7.8	15.7	5.2	3.7	21.8	9.1	6.7
		65	72		Dec. 30	22	24.4	8.1	6.0	12.0	16.4	9.7	9.7	20.3	6.8	5.0	25.9	10.8	9.8
	First-year stubble:				*1933*														
2	Before storage	66	71		Nov. 15	7	7.5	5.1	6.4	6.3	6.4	4.9	7.0	13.0	7.1	11.1	23.1	11.0	16.7
		66	71		Nov. 22	14	11.4	6.9	10.5	9.5	6.3	3.4	8.4	19.2	10.0	16.1	31.0	13.7	22.6
		66	71		Nov. 29	21	21.0	13.2	14.9	16.1	17.1	10.7	11.4	27.5	18.6	26.1	45.1	27.2	36.5
	After storage	66	71		Nov. 15	7	13.0	3.7	4.7	6.9	15.0	6.4	2.8	28.9	10.4	10.2	54.3	18.0	14.3
		66	71		Nov. 22	14	20.2	8.5	9.3	12.1	19.8	9.0	6.0	44.8	19.6	19.7	80.4	31.0	27.6
		66	71		Nov. 29	21	25.6	13.8	13.5	17.3	27.3	13.0	8.5	41.0	24.0	27.5	72.2	30.5	38.1
	Second-year stubble:				*1934*														
3	Before storage			Shade	Nov. 9	7	10.8	6.9	7.8	8.4	8.1	5.5	6.8	43.0	31.3	30.5	74.1	47.8	44.7
				do	Nov. 16	14	18.2	12.5	13.1	14.4	14.2	11.7	13.0	53.2	42.4	40.3	90.0	64.6	59.2
	After storage			do	Nov. 9	7	11.9	6.2	6.2	7.9	16.2	6.7	4.8	45.7	34.8	21.9	83.6	54.0	31.9
				do	Nov. 16	14	23.6	10.1	8.2	13.4	24.9	9.5	7.4	61.7	50.9	39.0		78.3	57.3
4	Before storage			do	Dec. 5	7	9.9	4.5	5.3	6.4	8.0	2.4	4.1	14.3	3.9	4.9	23.6	4.9	6.5
	After storage			do	do	7	14.4	2.7	1.7	5.8	10.9	4.3	2.3	20.6	.5	.6	33.0	.6	1.2

CO. 290, FIRST-YEAR STUBBLE

Experiment	Time of sectioning cane	Temperature	Relative humidity	Open shed	Date of analysis	Duration of storage	Weight loss Top third	Weight loss Middle third	Weight loss Bottom third	Weight loss Entire stalk	Brix Top third	Brix Middle third	Brix Bottom third	Sucrose in juice Top third	Sucrose in juice Middle third	Sucrose in juice Bottom third	Available sucrose Top third	Available sucrose Middle third	Available sucrose Bottom third
					1936														
5	Before storage			Shade	Nov. 4	5	7.7	5.3	5.0	5.9	4.9	5.4	5.4	19.2	4.9	3.2	38.3	8.2	5.1
				do	Nov. 9	10	11.0	7.4	6.9	8.3	5.1	6.7	5.4	38.0	14.4	9.7	75.1	23.6	14.2
				do	Nov. 16	17	13.7	10.7	10.3	11.4	11.2	8.5	9.7	32.5	17.3	15.0	68.2	27.1	22.5
	After storage			do	Nov. 4	5	9.7	3.2	1.5	4.6	5.3	1.7	1.2	27.8	6.6	2.5	55.1	10.1	+1.4
				do	Nov. 9	10	11.4	4.2	3.1	6.2	8.9	1.5	.6	37.5	16.7	9.6	77.5	26.4	13.6
				do	Nov. 16	17	16.2	8.0	3.7	8.9	18.9	7.7	3.3	32.5	18.7	10.3	70.4	31.0	15.8



GURE 2.—Loss in weight, increase in Brix, and drop in apparent purity in the top-, middle-, and bottom-third portions of the sugarcane stalk sectioned before and after storage: *A*, P. O. J. 36–M, second-year stubble, stored from November 2 to 16, 1934; *B*, Co. 290, first-year stubble, stored from October 30 to November 16, 1936. The cane of both varieties and treatments was stored in the shade

ABLE 5.—*Total loss of sucrose from sections of cane sectioned before storage, from those sectioned after storage, and from the whole stalk*

Variety	Conditions of storage			Date of analysis	Duration of storage	Cane sectioned before storage				Cane sectioned after storage				Whole stalk			
	Temperature	Relative humidity	Open shed			Yield of sucrose per ton of cane	Loss of sucrose		Loss in weight	Yield of sucrose per ton of cane	Loss of sucrose		Loss in weight	Yield of sucrose per ton of cane	Loss of sucrose		Loss in weight
	°*F.*	*Percent*			*Days*	*Pounds*	*Pounds*	*Percent*	*Percent*	*Pounds*	*Pounds*	*Percent*	*Percent*	*Pounds*	*Pounds*	*Percent*	*Percent*
O. J. 36–M, first-year ubble.	66	71		*1933* Nov. 8	0	157.4				157.4							
				Nov. 15	7	132.5	24.9	15.8	6.3	122.5	34.9	22.2	6.9				
				Nov. 22	14	124.4	33.0	21.0	9.5	98.5	58.9	37.4	12.1				
				Nov. 29	21	102.7	54.7	34.8	16.1	89.4	68.0	43.2	17.3				
). J. 36–M, second-year ıbble.			Shade	*1934* Nov. 2	0	172.0				172.0							
			do	Nov. 9	7	84.3	87.7	51.0	8.4	87.9	84.1	48.9	7.9				
			do	Nov. 16	14	57.5	114.5	66.6	14.4	44.4	127.6	74.2	13.4				
290, first-year stubble			Shade	*1936* Oct. 30	0	121.8				121.8				120.7			
			do	Nov. 4	5	108.6	13.2	10.8	5.9	109.2	12.6	10.3	4.6	114.0	6.7	5.6	4.0
			do	Nov. 9	10	90.3	31.5	25.0	8.3	89.1	22.7	18.6	6.2	97.8	22.9	19.0	5.8
			do	Nov. 16	17	85.1	36.7	30.1	11.4	87.0	34.8	28.6	8.9	88.2	32.5	26.9	8.8

Contrary to expectation, there was a greater drop in purity during storage (tables 2 and 3 and figs. 1 and 2) in the top third of the stalk of cane stored intact than in the top third stored as a section, and the reverse tended to be true of the bottom third. The relationship was very significantly true for P. O. J. 36–M, but it was less significantly so for Co. 290. The percentage of loss of sucrose in juice from P. O. J. 36–M (table 4) showed the same relationship as did the drop in purity. During the first period of storage there was a much greater loss of sucrose in Co. 290 in the top third of the stalk stored whole than in the top third stored as sections, but by the end of the second and third periods it was equal or slightly less. During most of the periods of storage there was a greater loss of sucrose in the bottom third of cane stored as sections than in the bottom third stored as whole stalks. Associated with these trends of inversion of sucrose in the top and bottom sections of the cane stalk, was a greater loss of moisture and increase in Brix in the top third of cane stored as whole stalks than in the top third stored as sections and in the bottom third of cane stored as sections than in the bottom third stored as whole stalks (table 4 and figs. 1 and 2). It would seem, therefore, that the loss of moisture in this instance, as in others, was a determining factor in bringing about inversion of sucrose. An explanation of the greater loss of moisture during storage in the top portion of an intact stalk of cane than in the top third of the stalk stored as sections and of the reverse tendency in the two treatments of the bottom third of the stalk is tentatively offered. The data indicate that there was a movement of water from the top to the bottom part of the stalk when intact and that this movement involved a greater loss of moisture from the top third than would have been the case if it had been severed from the remainder of the stalk. This movement of moisture may be accounted for by the difference in the osmotic pressure of the different parts of the cane stalk. Freezing-point data obtained in connection with varieties P. O. J. 36–M, Co. 281, and Co. 290 indicate that the osmotic pressure of the bottom and middle third of the stalk is always greater than that of the top third and is generally greater in the bottom than in the middle third.

In cane stored as whole stalks the percentage of loss of moisture and the increase in Brix tended to be greatest in the top third, next greatest in the middle third, and least in the bottom third. Such a trend might have been expected because of the physical difference in the three sections. The drop in purity (tables 2 and 3) and the percentage of loss of sucrose in juice and of available sucrose tended to follow the same trend (table 4 and figs. 1, 2, and 3), particularly during the early period of storage. The sectioning of the cane before storage disarranged the trend in moisture loss and increase in percentage of Brix; a similar disarrangement occurred or tended to occur with the drop in purity and the percentage of loss of sucrose in juice and of available sucrose. This disarrangement would seem to have resulted from the interruption of the movement of moisture from the top to the bottom part of the cane stalk, thus diminishing the loss of moisture in the top third and increasing it in tbe bottom third. In all five experiments this shift in loss of moisture resulted in a greater loss of moisture and consequently a greater increase in Brix in the top and bottom sections than in the middle section. There was a

corresponding shift in the drop in purity and percentage of loss of sucrose, and in three of the experiments these values were greater in the top and bottom sections than in the middle section. The increase in invert sugars in the different sections of both treatments in the two experiments in which they were determined (table 2) indicated the same trends as did the drop in purity and percentage of loss of sucrose. In most instances (table 2) a loss of total sugars

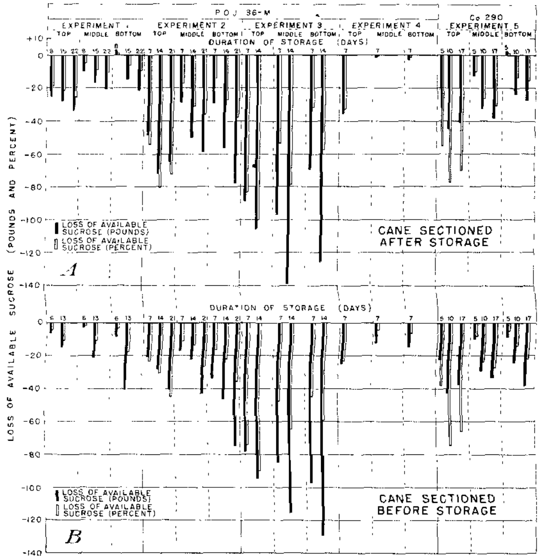


FIGURE 3.—Loss of available sucrose in pounds and in percentage as related to whole-stalk samples sectioned into one-third lengths after storage and then analyzed (*A*) and to samples sectioned before storage and analyzed after storage (*B*).

was indicated. This loss of sugars is regarded as having resulted from respiration.

There was a slight tendency for the percentage of increase in Brix as related to moisture loss to be accentuated in the top third of cane stored as whole stalks as compared with that in the top third of cane stored as sections. This tendency was reversed in the two treatments of the bottom sections. These observations suggest the possible movement of solids in cane stored as whole stalks and, in view of the greater drop in purity in the top third of this cane, of invert sugars

rom the bottom to the top part of the stalks, or a greater consump-ion of solids by respiration in the top third of cane sectioned before torage. It is hardly conceivable that there would be a movement of nvert sugars, for they would have to move against a gradient of oncentration. It seems possible that, because of additional wound-ng in the top sectioned before storage, respiration may have been ccelerated. It is also possible that some other conditions may be esponsible for the apparent accumulation or disappearance of solids.

Thus far the rate of loss of sucrose as measured by the drop in urity and the percentage of loss of sucrose in juice and of available ucrose only have been considered. The actual loss of sucrose and he loss of available sucrose are matters of practical importance to he sugarcane planter. Although in a given case the rate of loss of ucrose may be greater in the top third of the sugarcane stalk than n the bottom third, the number of pounds lost may be greater in the bottom third than in the top third (fig. 3). This will be appreciated when it is realized that rate or percentage loss of sugar is dependent upon the initial concentration of sucrose present in the different sections.

SUMMARY

The data presented relate to inversion of sucrose and to changes in noisture content, and in Brix in the top-, middle-, and bottom-third engths of the sugarcane stalk when sectioned into thirds and stored ind when stored intact and sectioned after storage. In two exper-ments cane of both treatments was stored under controlled conditions of temperature and humidity, and in three experiments it was stored inder dry conditions in the shade.

There was less inversion of sucrose in all samples stored at high elative humidities than in corresponding samples stored at low rela-ive humidities.

In general, the rate of inversion of sucrose was correlated with the ate of loss of moisture.

In most instances there was more total loss of moisture and inversion of sucrose from the three sections in samples sectioned before storage han in samples sectioned after storage. The exception seemed to be lue to the lack of uniform exposure of samples to the evaporating ower of the air.

In cane stored as whole stalks there tended to be a gradient in the ercentage of loss of moisture, percentage of increase in Brix, drop in urity, and percentage of loss of sucrose; these changes being greatest n the top, next greatest in the middle, and least in the bottom section.

In cane sectioned before storage this gradient was disarranged so hat these changes tended to be greater in the top and bottom sections han in the middle section.

In the experiments in which the variety P. O. J. 36-M was used here was greater inversion in the unsevered top third of the stalks han in the severed top third. On the contrary, during the early peri-ds of storage there was greater inversion in the severed bottom third han in the unsevered bottom third with the difference decreasing and ometimes disappearing with lapse of time. The behavior of the ariety Co. 290 was similar to that of P. O. J. 36-M except that the lifferences were not so marked and after the first period of storage

here was a decrease in the difference in the top sections instead of in he bottom sections.

Correlated with these trends of inversion of sucrose in the top- and ottom-third sections of the sugarcane stalk was a greater loss of noisture and increase in Brix in the top third of cane stored as whole talks than in the top third stored as sections and in the bottom third f cane stored as sections than in the bottom third stored as whole talks.

Because of the difference in concentration of sucrose in the different arts of the sugarcane stalk, the actual loss of sucrose may not correpond to the rate of loss. It is only when the rate is much greater in he sections with the lower concentrations that these sections show the reatest loss of sucrose.

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SALT BALANCE IN IRRIGATED AREAS [1]

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INTRODUCTION

It is generally recognized that the use of saline irrigation water involves the danger that soluble salts may accumulate in the irrigated soil to its detriment. The artificial drainage of irrigated lands thus has two objectives: (1) The removal of surplus water, whether surface or subsoil accumulations, and (2) the removal of surplus dissolved salts that might otherwise accumulate in the soil solution of the root zone or in the subsoil water immediately below the root zone. The effectiveness of a drainage system is to be judged by the conditions of crop growth and by field observations made to ascertain not only whether the surplus water is being removed, but also whether the drainage water is carrying away approximately as much dissolved salt as is being carried to the land by the irrigation water.

The objectives of the present paper are to describe methods and results (1) of field observations made to ascertain the quantities of dissolved salts carried to and removed from certain representative irrigated areas and (2) of a salt-balance experiment made at the Rubidoux Laboratory, Riverside, Calif.

In recording field observations, the relationship of salt input to salt output is designated as the salt balance for the area. If the mass of the salt input exceeds the mass of the salt output the salt balance is regarded as adverse, because this trend is in the direction of the accumulation of salt in the area and such a trend is manifestly undesirable

To illustrate the use of these methods of determining the trend o salt-balance conditions, three irrigated areas have been selected two of which are irrigated from the Rio Grande in the vicinity of E Paso, Tex., and the third from the Colorado River in the vicinity of Yuma, Ariz. These areas were selected for investigation because in each case the salinity of the irrigation water is relatively high, averaging approximately 1 ton of dissolved salts in an acre-foot of water and because in each case it has been possible to measure the volume of input as irrigation water and the volume of output as drainage water and to obtain representative samples of each for analysis.

The data for the years of record for each irrigated area are presented in this paper. They show the trend of salt-balance conditions for each area not only in respect to the total dissolved solids but also

[1] Received for publication April 10, 1940. The data presented herewith concerni..g the salt balance o the Mesilla Valley and the El Paso Valley have been obtained through a cooperative investigation conducted by the Bureau of Reclamation, U. S. Department of the Interior, and the Division of Irrigation Agriculture, Bureau of Plant Industry, U. S. Department of Agriculture. The data as to the discharg of the stream at the gaging stations have been reported by the Bureau of Reclamation and the U. S. Section International Boundary Commission, United States and Mexico; and the water samples, collected at leas once a week at each gaging station, have been taken by the same agencies. The analyses of the wate samples and the computation and compilation of the findings have been made by the Bureau of Plan Industry. This cooperative investigation has been carried on since February 1930, but because the sampling program during that year was not continuous, the period of the present report begins with January 1931

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n respect to each of the seven more important salt constituents. It s obvious, however, that these data as to salt input and salt output or the irrigated area as a whole may not give a true picture of the rend or rate of change of salt conditions in the root zone of the soil, ind it is the conditions within the root zone that are critical in deternining crop production. Information as to the total quantity of the lissolved salts or the quantity of each constituent of the input and f the output is not sufficient to show the trend of conditions in the oot zone of the soil. Information is needed also as to the destination f the input salts: how much is absorbed by the crop plants and how nuch passes through the root zone into the subsoil water. Similarly, nformation is needed as to the origin of the output salts: how much s derived directly from the root-zone leaching and how much from he subsoil water that existed in the area prior to the period of record r even prior to the period of irrigation.

The scope of the present field investigation did not include any nquiry as to the volume of or the changes in the concentration of alts in the reservoir of subsoil water underlying any of the irrigated reas. The data reported must be taken as applying to each area as whole and not alone to conditions in the surface or root-zone horizon. for does this field investigation afford information as to what proortion of the dissolved salts carried in the irrigation water may be bsorbed by the crop plants. It has been assumed that this destinaon can account for only a small part of the total input when the salt oncentration of the irrigation water ranges up to 1,000 parts per illion, or more.

In order to supplement the field investigation and to obtain some formation as to the movement and destination of the salts applied a crop-producing soil with irrigation water, an experiment was set p and conducted for more than 4 years. The conditions of the perinment were such that the volume of irrigation input and of rainage output could be measured precisely; also, the concentration dissolved salts in the input and output solutions was carefully etermined and the crop growth (alfalfa) was harvested and weighed. rom the data of this experiment, herein reported, it can be shown ow much of the input water may be dissipated by surface evaporation id plant transpiration and how much must be allocated to root-zone aching in order to prevent salt accumulation in the soil. Furtherore, it can be shown what quantity of water was dissipated by aporation and transpiration for each unit quantity of crop proiced, and finally how much of the input salt was recovered in the ainage or percolate when the soil was leached to its original salt ntent. The difference between the mass of salts in the irrigation put and in the drainage output indicates the quantity of salts sorbed by the crop plants.

It is not to be inferred that the data reported from this experiment ay be applied quantitatively to field conditions. With the same or nilar climatic conditions, it is to be expected that the use of land r one group of crops would give results differing quantitatively from sults obtained from its use for another and different group of crops. so it is to be expected that the same or similar groups of crops uld show different quantitative results when grown under different matic conditions. Notwithstanding these qualifications, it is

believed that these data, both from field observations and from a controlled experiment, may lead to a better understanding of the factors and relationships that exist in an irrigated area in respect to dissolved or soluble salts and to the problem of the adequate drainage of irrigated land.

FIELD INVESTIGATIONS

METHODS

Sampling and Analysis

The water samples involved in the present investigation were taken at gaging stations by the hydrographers responsible for measuring the stream discharges. At Leasburg Dam, on the Rio Grande, the hydrographer is employed by the Bureau of Reclamation, United States Department of the Interior. Samples were taken approximately once a week. Prior to November 1933 each sample was separately analyzed in detail. Beginning with November 1933, each weekly sample was analyzed only by determining its specific electrical conductance ($K \times 10^5 @ 25^\circ$ C.) and then the four or five samples for each month were made into a composite for detailed analysis. In making up the monthly composites the volume of the aliquot taken from each sample was proportional to the volume of the discharge it represented.

At the Courchesne (El Paso) gaging station and also at the Fort Quitman gaging station, the hydrographers are employed by the United States Section of the International Boundary Commission, United States and Mexico. The sampling and analytical program for these two stations has been the same as that at Leasburg Dam except that since September 1937 the hydrographer at the Courchesne station has taken samples daily, determining the conductance of each sample, and has then made up the monthly composites, contributing from each sample a volume proportional to the discharge represented. Each of these monthly composites has then been analyzed in detail.

For the Yuma Valley, samples of the irrigation supply were taken at the Yuma gaging station each week by a hydrographer employed by the Bureau of Reclamation. Each sample was analyzed separately. Samples of the drainage water discharged from the Yuma Valley were taken weekly at the pumping plant located at the international boundary, at the south end of the valley. Prior to January 1935 each weekly sample was analyzed in detail. Subsequently, monthly composites were made up after the conductance of each individual sample had been determined, and these monthly composites were analyzed.

The methods of analysis used were those currently in use at the Rubidoux Laboratory, Riverside, Calif.[2]

Computation

In making the computations of the salt burden of a stream at a gaging station or of the salt-balance conditions of an irrigated area, the essential data include (1) the volume of water passing a given point in a given period of time and (2) the concentration and com-

[2] Methods and Analysis Used in the Rubidoux Laboratory, Riverside, California. Ed. 2, rev 1938. [Mimeographed.]

position of the salts or salt constituents dissolved in the water. Th rate of discharge of an irrigation stream is usually reported in term of cubic feet per second of time (c. f. s.), which values may be con verted into terms of acre-feet per day by multiplying them by th factor 1.98. The hydrographic reports of stream discharge are usuall summarized to show discharge volumes as acre-feet per month. Ir the present computations the data of salt concentration and composi tion were also determined by months, either by the analysis of a monthly composite sample or by computing the mean of the analyse of the several individual samples taken during the month.

The data of the salt burden of an irrigation stream include (1) th quantity of total dissolved solids or of total salts and (2) the quantit of each of the salt constituents as determined by the detailed analysis In this paper a distinction is made between the "total dissolvec solids" and the "total salts." The value for total dissolved solids is computed from the results of a gravimetric determination in whicl a measured volume of a filtered aliquot of the water sample is evap orated and the residue is dried to constant weight at 105° C. Th value for total salts is obtained by taking the sum of the gravimetric equivalents of the ions determined by the detailed analysis. These gravimetric equivalents are the products of the values for milligram equivalents per liter by the combining weight of each ion referencec to hydrogen as 1. In computing the gravimetric equivalent of the bicarbonate ion the combining weight is taken as 30.5.

In general the value obtained for total dissolved solids is somewhat greater than the value for total salts. The difference may be due in part to (1) some water of crystallization held by the salt residues, (2) some silica or other constituents not included in the detailed analysis, and (3) some dissolved organic matter that may pass the filter. In the waters of the Rio Grande and the Colorado, the concentrations of total salts are usually 90 to 92 percent of those of total dissolved solids. When the concentration values for total salts or total dissolved solids are reported as parts per million (equivalent to milligrams per liter), these may be converted into tons per acre-foot by multiplying by the factor 0.00136. This factor is derived from the assumption that an acre-foot of water weighs 2.72 million pounds.

The concentrations of the seven salt constituents are reported as milligram equivalents per liter. In order to convert these values into their equivalents as tons per acre-foot, a factor is used that is the product of the combining weight of the ion by the factor 0.00136. The factors used in these conversions are shown in table 1.

Thus, if volume of discharge for a month was 10,552 acre-feet and the calcium concentration was 4.94 mg. equiv. per liter, the computation for the tonnage of calcium in that volume of water would be:

$$4.94 \times 0.0272 \times 10{,}552 = 1{,}418 \text{ tons.}$$

The data for a gaging station were thus computed for each constituent for each month of a year and were tabulated and summarized. These annual summaries were then used in computing the weighted mean concentration and composition of the dissolved salts carried in the water.

TABLE 1.—*Factors for converting concentration values of salt constituents from milligram equivalents per liter to tons per acre-foot*

Constituent	Combining weight	Factor (=combining weight× 0.00136)
Calcium	20	0.0272
Magnesium	12.15	.01652
Sodium	23.0	.03128
Carbonate and bicarbonate	30.5	.04148
Sulfate	48.0	.06528
Chloride	35.5	.04828
Nitrate	62.0	.08432

THE MESILLA VALLEY

DESCRIPTION

The area here designated as the Mesilla Valley includes that section of the flood plain of the Rio Grande between the Leasburg Dam near old Fort Selden, N. Mex., and the International Dam near El Paso, Tex. It is approximately 55 miles long and generally less than 5 miles wide. Its area is about 110,000 acres, of which approximately 80,000 are currently irrigated. Of the irrigated area, about 50,000 acres are devoted to cotton and 20,000 to forage crops, chiefly alfalfa. The land surface is about 3,800 feet above sea level. The mean annual temperature is 60° F. The mean annual precipitation is about 8.5 inches, most of which occurs during the late summer.

The irrigation supply for the valley is diverted from the Rio Grande, partly at Leasburg Dam, located in the NE¼ sec. 10, T. 21 S., R. 1 W., of the New Mexico principal meridian, and partly at Mesilla Dam, located in the NW¼ sec. 13, T. 24 S., R. 1 E. The water entering the valley by the river is measured at Leasburg Dam. There are no important tributaries to the river between Leasburg Dam and El Paso; consequently, the contribution of water other than that from the river comes from the precipitation falling on valley land and on slopes adjacent to the valley. The irrigated lands of the valley are served by a system of distributing canals fed from one or the other of the diversion dams on the river. They are served also by a system of open drains, aggregating 211 miles in length, that discharge by gravity into the river above El Paso. The water leaving the valley by the river is measured at the Courchesne gaging station, near International Dam and below the lowest outlet from the valley drains. Thus it is possible to measure and to sample the water both as it enters and as it leaves the valley by the river.

Not all of the water carried by the river into the valley is diverted into the canals. The river brings in annually about 750,000 acre-feet; the annual precipitation in the valley land constitutes about 75,000 acre-feet. The annual outflow from the valley passing the Courchesne gaging station is about 500,000 acre-feet, of which about 200,000 acre-feet is contributed from the valley drains. Thus it may be assumed that the annual diversions from the stream may amount to 450,000 acre-feet, i. e., to the difference between the annual inflow and the annual outflow, 250,000 acre-feet, plus 200,000 acre-feet returned to the stream through the drains. The annual dissipation

of water from the valley lands may be estimated at 325,000 acre-feet, i. e., the 250,000 acre-feet of stream depletiou plus 75,000 acre-feet of precipitation.

Salt Balance

The essential facts in respect to the conditions of salt balance in an irrigated area involve at least two comparisons: (1) A comparison of the input with the output of the total dissolved salts and (2) a comparison of the input with the output of each of the more important constituents of these dissolved salts. It must be recognized also that these salts and salt constituents move into or away from an irrigated area only as they are dissolved in and carried by the water of irrigation and drainage. Consequently, a report on salt-balance conditions must include consideration of the volume of water moving into and the volume of water moving away from an irrigated area and the concentration of the dissolved salts in these waters. In table 2, the data presented fall into five categories: (1) The first three lines of the table relate to the annual volumes of water (a) moving into the area, (b) moving out from the area, and (c) dissipated from the area as evaporation and transpiration; (2) the next two lines give the concentration of dissolved salts in (a) the inflowing water and (b) the outflowing water; (3) the next two lines give the tonnage of total dissolved salts annually carried by the water (a) into the area and (b) away from the area; (4) the next line gives the ratio, as percentage, of the annual volume of outflow to the annual volume of inflow; and (5) the last line gives the cumulative tonnage of dissolved salts removed annually, for the period of record, from the area; i. e., the excess of salts removed as compared with salts brought in.

Table 2.—*Salt balance, Mesilla Valley, 1931–37*

Item	1931	1932	1933	1934	1935	1936	1937	Mean
Water:								
Input .... acre-feet	738, 000	814, 000	824, 000	768, 230	632, 400	693, 260	740, 770	744, 380
Output .... do	518, 000	567, 240	609, 180	508, 480	259, 910	473, 740	536, 240	496, 113
Dissipated .... do	220, 000	246, 760	214, 820	259, 750	372, 490	219, 520	204, 530	248, 267
Salts:[1]								
Input .... tons	621, 655	697, 204	588, 367	642, 955	566, 809	550, 025	527, 970	599, 369
Output .... do	655, 801	736, 661	647, 509	589, 594	528, 576	531, 410	567, 002	608, 076
Concentration:								
Input .. tons per acre-foot	0. 842	0. 857	0. 714	0. 837	0. 896	0. 794	0. 713	0. 805
Output .... do	1. 266	1. 209	1. 063	1. 159	2. 034	1. 122	1. 057	1. 226
Water output[2] .... percent	70. 2	69. 7	73. 9	66. 2	41. 1	68. 3	72. 4	66. 6
Cumulative salts removed .... tons	34, 146	73, 603	132, 745	79, 384	41, 151	21, 936	60, 968	8. 707

[1] Sum of ions determined. [2] Percentage of input.

It will be observed from table 2 that for 7 years, 1931–37 inclusive, the mean annual inflow of water was 744,380 acre-feet. The mean annual outflow was 496,113 acre-feet, or 66.6 percent of the inflow. The mean annual difference, i.e., stream water dissipated, was 248,267 acre-feet. The mean concentration of the inflowing water was 0.805 ton per acre-foot, and that of the outflowing water 1.226 tons per acre-foot. These concentrations, expressed as parts per million, or milligrams per liter, are 592 and 901, respectively. The mean annual tonnage of dissolved salts carried into the area was 599,369, and the tonnage

carried out was 608,076, indicating an annual net removal of 8,707 tons

In order to present a more detailed consideration of salt-balanc conditions in the Mesilla Valley, the facts concerning the severa constituents of the dissolved salts are given in table 3. The firs two and the last lines of table 3 include the mean values from tabl 2 in respect to the input and output of stream water, of total dissolve salts, and of concentration of dissolved salts in the water. Th remaining lines relate to the several constituents of the dissolved salts For each of these the tonnage of input and of output is given, followe by the ratio of the output to the input expressed as a percentage. I will be noted that for the total salts the output was 101.5 percent o the input.

TABLE 3.—*Balance of salt constituents, Mesilla Valley; means for 1931–37*

Item	Input	Output	
	Acre-feet	*Acre-feet*	*Percent* [1]
Water	744,380	496,113	66.
	Tons	*Tons*	
Salts [2]	599,369	608,076	101.
Salt constituents:			
Calcium (Ca)	80,558	69,686	86.
Magnesium (Mg)	17,557	15,468	88
Sodium (Na+K)	101,985	122,639	120
Bicarbonate (HCO_3+CO_3)[3]	93,936	80,540	85
Sulfate (SO_4)	233,419	207,950	89
Chloride (Cl)	71,306	111,122	155.
Nitrate (NO_3)[4]	1,418	1,461	103
	Tons per acre-foot	*Tons per acre-foot*	
Concentration	0.805	1.226	152

[1] Percentage of input.
[2] Sum of ions determined.
[3] Computed as CO_3.
[4] For 1935 to 1937 only.

It is obvious at once that the output percentages of the salt cor stituents differ from that for the total salts. In other words, the con position of the salts in the outflowing water was different from th in the inflowing water. From this it follows that, while for the Mesil Valley the annual output of total salts exceeded the annual inp slightly, this was not the case with four of the seven salt constituent With these four, viz, calcium, magnesium, bicarbonate, and sulfat the output percentages ranged from 85.7 to 89.1. On the other han the output of sodium was 120.3 percent of the input; of chlorid 155.8 percent; and of nitrate, 103 percent.

The fact that the composition of the salts in the outflowing strea water differed from that of the inflowing water is not surprisin The subsoil of the valley land is saturated with water, some of whi is very salty, and the composition of these salts differs from that of t salts of the river water. The salts of the subsoil water general contain higher proportions of sodium and of chloride. These tw constituents form a salt of high solubility, which may remain in concentrated solution after the less soluble salts, such as the sulfat and carbonates of calcium and magnesium, have been precipitate This subsoil water with its dissolved salts constitutes a large part the drainage from the irrigated land, and this drainage makes up abo

10 percent of the volume of the outflowing water. Thus it is to be expected that the composition of the salts of the outflowing water would differ from that of the salts carried by the inflowing water.

THE EL PASO VALLEY

DESCRIPTION

The area here designated as the El Paso Valley includes the irrigated lood plain on both sides of the Rio Grande between El Paso, Tex., md the Fort Quitman gaging station, which is located about 65 miles lownstream from El Paso. This area includes not only the irrigated section that lies along the river in El Paso County, Tex., but also he section on the Mexican side of the international boundary, mown locally as the Juarez Valley, and also the section in Hudspeth County, Tex. The total area on the American side of the international oundary is slightly less than 100,000 acres, of which about 60,000 cres are irrigated. There is probably a smaller area of the Mexican md, both total and irrigated, but data are not available as to this rea. The allocation to crops on the American lands is similar to nat of the Mesilla Valley. The elevation is only slightly lower, nd the climatic conditions are much the same.

The irrigation water for lands on both sides of the river was formerly iverted at International Dam, Tex., located just west of El Paso. Recently, however, a new dam has been constructed about 2 miles pstream and just above the international boundary, and the American nds have been served from the new dam. There is one supplemental iversion for the American lands, known as the Riverside Heading, ocated on the river about 2 miles south of Ysleta, Tex., and another rther down at Fabens, Tex. The lands of the Hudspeth district re irrigated in part with water diverted for the lands in El Paso County, but not there used, and in part with water from the river iverted about 3 miles above Fort Hancock, Tex. The irrigated ands in El Paso County are served by 210 miles of drains, which discharge annually about 133,000 acre-feet of water, some of which is e-used for irrigation. The stream water entering the El Paso Valley s measured at the Courchesne gaging station, just above International Dam, and the total outflow is measured at the Fort Quitman gaging tation.

Most of the river water that enters El Paso Valley is diverted and sed for irrigation. While there are no important tributaries joining ie main stream between El Paso and Fort Quitman, there are several rroyos that drain the higher lands bordering the valley, and after ie more intense rains that occur occasionally in late summer these rroyos carry floodwaters for a short time. These floodwaters contribute very little to the irrigation supply and probably carry very ttle salt. They do, however, contribute to the volume of water assing the Fort Quitman gage and reduce its salt concentration for ort periods. In view of uncertainties as to land areas and volume run-off water from adjacent higher land, no estimate was made of e water contributed to the valley by precipitation nor of the total lume dissipated from the irrigated land.

Salt Balance

The data covering stream discharge and quality of water for the El Paso-Jaurez Valley have been obtained as a part of the cooperative investigation described above. The data for total salts are given in table 4. The data for the inflowing water for the El Paso Valley are the same as those for the outflow from the Mesilla Valley. For the 7-year period 1931–37, the mean volume of stream inflow was 496,113 acre-feet and the mean outflow 173,219 acre-feet, or 34.9 percent of the inflow. The difference between these values, 322,894 acre-feet, may be taken as the net stream depletion, or water dissipated in addition to the quantity of rainfall dissipated. It will be noted that the volume of output expressed as percentage of input ranged widely, from 20.1 in 1934 to 55.9 in 1935. Following the dry year of 1934, the water supply in Elephant Butte Reservoir was low, so that the volume available for the El Paso Valley in 1935 was below normal. There were also local torrential rains in the late summer of 1935 that contributed to the volume of run-off passing Fort Quitman. During the last 4 months of 1935, the discharge at Fort Quitman was 101,100 acre-feet as compared with 24,200 acre-feet for the corresponding period of 1934.

Table 4.—*Salt balance of El Paso Valley, 1931–37*

Item	1931	1932	1933	1934	1935	1936	1937	Mean
Water:								
Input .... acre-feet	518, 000	567, 210	609, 180	508, 480	259, 910	473, 740	536, 240	496, 11
Output .... do	212, 000	211, 120	213, 790	102, 360	145, 380	149, 590	178, 290	173, 21
Dissipated .... do	306, 000	356, 120	395, 390	406, 120	114, 530	324, 150	357, 950	322, 89
Salts:[1]								
Input .... tons	655, 801	736, 661	647, 509	589, 594	528, 576	531, 410	567, 002	608, 07
Output .... do	637, 231	597, 996	542, 285	285, 963	289, 919	402, 520	476, 642	461, 79
Concentration:								
Input..tons per acre-foot	1. 266	1. 299	1. 063	1. 159	2. 034	1. 122	1. 057	1. 22
Output .... do	3. 006	2. 832	2. 537	2. 794	1. 994	2. 691	2. 673	2. 66
Water output[2] .... percent	40. 9	37. 2	35. 1	20. 1	55. 9	31. 6	33. 2	34.
Cumulative salt residue tons	18, 567	157, 232	262, 456	566, 087	804, 744	933, 634	1,023,994	146, 28

[1] Sum of ions determined. [2] Percentage of input.

The mean salt concentration of inflowing stream water was 1.22 tons per acre-foot, as compared with the mean concentration of the outflow of 2.666. These values are equivalent, respectively, to 90 and 1,960 p. p. m. The mean annual input of dissolved salts was 608,076 tons, whereas the mean annual output was 461,794 tons indicating a mean annual residue of 146,282 tons, or a cumulative residue for the 7-year period of 1,023,994 tons. In respect to salt balance, conditions in the El Paso-Juarez Valley differed from those reported above for the Mesilla Valley. For the Mesilla Valley the output of dissolved salts exceeded the input by a mean of 8,707 tons a year, whereas for the El Paso Valley the input exceeded the output by 146,282 tons a year.

It is not to be inferred that the adverse salt balance of the El Paso-Juarez Valley, amounting to a million tons during the past 7 years, has been deposited uniformly in the irrigated soils of the valley. The

salt has been deposited. The known facts are that for the valley as a whole the input of dissolved salts has exceeded the output by nearly 150,000 tons a year. It should be pointed out, however, that the quantity of soluble and dissolved salts in the soil of the valley and in the subsoil water contained in the valley sediments is very large. An acre-foot of soil may contain as much as 5 tons of soluble salts without seriously impairing its productivity, and in the El Paso Valley the salt content of the deeper sediments, below the root zone, may be much higher than that.

The facts concerning the balance of salt constituents for the El Paso Valley are given in table 5, which shows the mean annual tonnage of the input and output of each of seven salt constituents. For only one of these, chloride, was the output in excess of the input. For the total salts the mean annual output was 75.9 percent of the input. For the salt constituents the output percentages of both sodium and chloride exceeded that value.

TABLE 5.—*Balance of salt constituents, El Paso Valley; means for 1931–37*

Item	Input		Output		
	Acre-feet	*Ton equivalents* [3]	*Acre-feet*	*Ton equivalents* [3]	*Percent* [1]
Water	496,113		173,219		34.9
	Tons		*Tons*		
Salts [2]	608,076		461,794		75.9
Salt constituents:					
Calcium (Ca)	69,686	3,484	41,309	2,065	59.3
Magnesium (Mg)	15,468	1,268	10,217	837	66.1
Sodium (Na+K)	122,639	5,332	111,654	4,855	91.0
Bicarbonate (HCO_3+CO_3)	80,540	2,641	28,247	926	35.1
Sulfate (SO_4)	207,950	4,332	108,702	2,265	52.3
Chloride (Cl)	111,122	3,130	161,447	4,548	145.3
Nitrate (NO_3) [4]	1,461	24	508	8	34.8
	Tons per acre-foot		*Tons per acre-foot*		
Concentration	1.226		2.666		217.5

[1] Percentage of input.
[2] *Sum of ions determined.*
[3] Ton equivalent represents the actua tonnage divided by the combining weight of each constituent. The sums of the ton equivalents of the cations and of the anions should be approximately equal if the tonnage computations have been correct.
[4] For 1935–37 only.

THE YUMA VALLEY

DESCRIPTION

The Yuma Valley lies in the extreme southwestern corner of Arizona, in the flood plain of the Colorado River. It contains about 50,000 acres of irrigable land, of which about 40,000 acres are cropped. The ground surface is only about 100 feet above sea level. It is protected from river overflow by a levee. The climate is warm and dry. Crops may be grown throughout the year; some of the land yields two crops in a single year. The annual precipitation is only 2 to 5 inches and is almost negligible as a contribution to the water requirements of crops. The crops chiefly grown are cotton and alfalfa, with some barley, grain sorghum, and winter-grown vegetables.

The irrigation water is diverted from the Colorado River at Laguna Dam, a few miles upstream from Yuma. It is carried by canal on the west side of the river to a siphon near Yuma, through which it passes

under the river to the eastern or Arizona side at the upper or north end of the Yuma Valley. Some of the water delivered into the distribution system of the Valley Division is lifted by pumps to irrigate land on the Yuma mesa. Another portion is wasted back into the river or across the international boundary into Mexico. However, the total quantity of water annually delivered through the siphon is measured, as are also the quantities pumped to the mesa or wasted outside the valley from the distributing canals. Thus there is available, by difference, the net quantity of water annually delivered to the distribution system of the Valley Division. Data as to the quality of this water are based on samples taken each week from the river at the Yuma gaging station.

The irrigated lands of the Yuma Valley are served by a system of open drains that collect subsoil water, together with some waste water from the canals, and discharge this drainage water into a sump at the lower end of the valley from which it is pumped over the levee to make its way back to the river in Mexico. The quantity of the drainage water is measured, and data as to its quality are based on samples taken each week at the pump intake. The difference between the annual net input and the annual drainage output is taken as the quantity annually dissipated from the valley by evaporation and transpiration. For the year ending September 30, 1938, the net input was 259,917 acre-feet and the drainage output was 57,095 acre-feet, leaving a difference of 202,822 acre-feet as the quantity dissipated from 50,000 acres of valley land.

Salt Balance

The data on the salt-balance conditions of the Yuma Valley fall into two categories. One of these (table 6) relates to the 10-year period 1929–38, and the other (table 7) to the year 1938 only. The reason for the distinction lies in the fact that prior to 1938 the data for volume of irrigation input were based on the quantities of water "delivered to farms" rather than on the net quantities of water delivered to the distribution system of the valley. In 1938, for the first time, the report of input included both the net quantity delivered to the valley and the quantity delivered to farms. For the year ending September 30, 1938, the net delivery to the valley was 259,917 acre-feet, while for the same period the quantity reported as delivered to farms was 113,659 acre-feet. These two values would indicate that during the year the loss from the distribution system, presumably by percolation and evaporation, was 146,258 acre-feet, or 56 percent of the net inpnt.

It seems obvious that the actual conveyance loss was not so great as is indicated by these values. The assumption is that the value for water delivered to farms is too low. In measuring farm deliveries the ditch riders tend to be liberal to the water user, and it is probable that the actual deliveries to farms exceed the reported deliveries.

The data for the 10-year period are given in table 6. Column 2 shows the quantities of water reported as annually delivered to farms. Column 3 shows the annual volume of drainage water pumped over the levee at the south end of the valley. The fourth column shows the differences between the values of columns 2 and 3. These difference values should not be taken as the quantities of water annually dissipated from the valley area of 50,000 acres, because the actual net

input of water has doubtless been much in excess of the quantity reported as delivered to farms. Columns 5 and 6 show the quantities of total dissolved solids in the water delivered to farms and in the drainage output, respectively. These values are computed from the residues of evaporated filtered samples and exceed by 9 or 10 percent the values for total salts obtained by taking the sum of the ions as determined by analysis.

TABLE 6.—*Annual volume of water "delivered to farms" in the Yuma Valley, annual volume of drainage output, and tonnage of total salts carried by each for the years ending Sept. 30, 1929–38*

Year	Delivered to farms	Drainage output	*Difference*	Total dissolved solids in— Water "delivered to farms"	Total dissolved solids in— Drainage
	Acre-feet	*Acre-feet*	*Acre-feet*	*Tons*	*Tons*
1929	106, 393	48, 344	58, 049	107, 503	108, 141
1930	100, 453	49, 158	51, 295	98, 685	98, 398
1931	100, 620	37, 394	63, 226	129, 224	71, 854
1932	95, 208	33, 477	61, 731	104, 139	64, 258
1933	99, 345	29, 975	69, 370	135, 436	60, 576
1934	108, 352	27, 169	81, 183	166, 498	59, 605
1935	110, 635	23, 574	87, 061	138, 828	58, 560
1936	119, 597	36, 830	82, 767	108, 797	82, 986
1937	115, 931	47, 000	68, 931	112, 152	102, 466
1938	113, 659	57, 095	56, 564	117, 624	121, 728

TABLE 7.—*Salt balance, Yuma Valley, for the year ending Sept. 30, 1938*

[Irrigable acres 49,278; cropped, 36,250]

Item	Input	Output	
	Acre-feet	*Acre-feet*	*Percent* [1]
Water	259, 917	57, 095	22. 0
	Tons	*Tons*	
Salts [2]	248, 428	113, 791	45. 8
Salt constituents:			
Calcium (Ca)	35, 552	11, 517	32. 4
Magnesium (Mg)	9, 113	4, 111	45. 1
Sodium (Na)	34, 895	23, 579	67. 6
Bicarbonate (HCO_3+CO_3)	26, 303	11, 698	44. 5
Sulfate (SO_4)	113, 771	30, 256	26. 6
Chloride (Cl)	28, 024	32, 542	116. 1
Nitrate (NO_3)	770	88	11. 4
	Tons per acre-foot	*Tons per acre-foot*	
Concentration	0. 956	1. 993	208. 5

[1] Percentage of input. [2] Sum of ions determined.

For the 10-year period, if comparison is made between the tonnage of total dissolved solids carried by the water delivered to farms and that carried by the drainage output, it will be seen that the cumulative adverse salt balance has been about 390,000 tons or 39,000 tons a year. However, this finding is probably too low because the total input of dissolved solids doubtless exceeded the quantity reported. It is believed that the quantities of total dissolved solids reported for the drainage water approximate closely the actual output.

It remains now to consider the data on salt balance for the year

which the net volume of irrigation water entering the Yuma Valley has been reported. The data for the year are shown in table 7. The volume of input for the year was 259,917 acre-feet; the volume of output was 57,095 acre-feet, or 22 percent of the input. The tonnage of total salts contained in the input was 248,428; that of the output was 113,791, or 45.8 percent of the input. The adverse salt balance for the year; i. e., input minus output, was 134,637 tons. The weighted mean concentration of salts in the input water was 0.956 ton per acre-foot; that of the drainage water was 1.993 tons per acre-foot.

The same table shows the tonnage values for each of seven salt constituents in the input water and in the drainage, together with the ratios of the output values to the input values, expressed as percentages of input values. These values show that of the seven constituents only the output percentages for sodium and chloride exceeded the percentage for total salts. It was only in the case of the chloride constituent that output tonnage actually exceeded the input tonnage, about 4,500 tons more chloride being found in the drainage than in the irrigation water.

With regard to the data concerning the nitrate constituent, table 7 shows an input of 770 tons of nitrate (NO_3) and an output of only 88 tons. Unfortunately the analyses did not include determinations of potassium and phosphorus, which, together with nitrogen, constitute the three major plant-food elements. It is evident that although the conditions of irrigation and drainage in the Yuma Valley are such as to remove actually more chloride than is brought in, the same conditions do not take out as much nitrate as is brought in. Furthermore, it is to be assumed that some nitrate is added to the soils of the valley by applications of manure and commercial fertilizer and also by the production of an extensive acreage of alfalfa.

A SALT-BALANCE EXPERIMENT

PROCEDURE

An experiment designed to yield information as to salt-balance conditions in the root zone of the soil was set up at the Rubidoux Laboratory, Riverside, Calif., in June 1934. It was designated experiment No. 28.[3] The equipment consisted of two galvanized-iron cans 15 inches in diameter and 20 inches deep, each having a tubular drainage outlet at the bottom. A layer of coarse sand, about 1 inch deep, was placed in the bottom of each can to facilitate drainage. On this was placed about 18 inches of soil, having a surface area of 1.35 square feet. The mass of dry soil in each can was 86.5 kg., and its texture was such that after several wettings in excess it held 20 liters of water after percolation ceased. The original soil contained very little soluble salts, and some of this was leached away in the preliminary wettings. It was estimated that at the beginning of the experiment, on June 28, 1934, each can contained approximately 10 gm. of dissolved salts in its 20 liters of water.

Alfalfa (*Medicago sativa* L.) seed was planted in the soil of each can, and in due course the plants became well established and grew vigorously. Figure 1 shows the cans used in the experiment and the growth of alfalfa 3 years after the experiment was started. The regimen of

[3] [illegible] in the conduct of this

irrigation has not been the same throughout the period of the experiment here reported, i. e., from June 28, 1934, to December 21, 1938. For certain periods the irrigation water used (the same water for both cans) contained dissolved salts in concentrations as high as 1.5 gm. per liter, or 1,500 p. p. m. For other periods the concentration of the input was much lower. The volumes of water used were sometimes not the same for both cans. For certain periods, the volume

FIGURE 1.—A crop of alfalfa grown in soil-filled cans in a salt-balance experiment at the Rubidoux Laboratory, Riverside, Calif.

applied to one can would be so large that the volume of percolate would be 25 percent of the volume of input while the volume applied to the other can would yield percolate of 10 percent or less of the volume of input.

Several times during the course of the experiment, irrigation water of low salinity was applied to both cans in large quantities in order to leach out the dissolved salts that had previously accumulated in the soil. Such a period of leaching preceded the conclusion of the

percolate obtained just prior to December 21, 1938, it was estimated that each can contained approximately 22 gm. of dissolved salts in its 20 liters of water held in the soil.

RESULTS

DATA FOR 1934–38

The data of the experiment make it possible to show (1) the volume of water and the mass of dissolved salts applied to each can during any period of time; (2) the volume of water and the mass of dissolved salts obtained as percolate from each can during any period of time; (3) for each period of time allocated to the growth of each successive crop of alfalfa, the volume of water dissipated from each can by evaporation and transpiration (volume of input water minus volume of percolate); this volume of water (expressed as liters), divided by the value for dry weight of the crop (expressed as grams), gives the water requirement for the crop; and (4) for each period of time between the successive thorough leachings, the mass of salts absorbed by the plants or by the soil or decomposed and dissipated (mass of dissolved salts contained in input water minus mass contained in percolate).

The record of the experiment as to water and salts for both cans for the whole period, from June 28, 1934, to December 21, 1938, is shown in table 8. During that period the total input of water was 5,490 liters, containing 5,443 gm. of dissolved salts. This indicates a mean concentration of 0.991 gm. per liter. For the same period the volume of the percolate was 1,237 liters, or 22.5 percent of the volume of the input. This volume of percolate contained 4,675 gm. of dissolved salts, or 85.9 percent of the input mass, and had a mean concentration of 3.779 gm. per liter. The volume of water dissipated as evaporation and transpiration, i. e., the volume of input minus the volume of percolate, was 4,253 liters. The mass of salt absorbed or dissipated, also the difference between input and output, was 768 gm., which includes 24 gm. of additional residue in the cans on December 21, 1938. This mass of salts, with reference to the volume of water dissipated, *indicates a mean concentration of 0.175 gm. per liter.*

TABLE 8.—*Record of experiment 28, from June 1934 to December 1938 and from Mar. 16 to Dec. 21, 1938, showing volumes, total salts, and concentrations of input, output, and residue*

Years and other items	Input	Percolate	Evaporation and transpiration
1934–38			
Solution volume……liters	5,490	1,237	4,253
Total salts……grams	5,443	4,675	[1] 768
Mean concentration……grams per liter	0.991	3.779	0.175
1938			
Solution volume……liters	1,438.3	389.4	1,048.9
Total salts……grams	1,609.1	1,397.1	[1] 212.0
Mean concentration……grams per liter	1.119	3.588	0.179

[1] Includes 24 gm. residue in the soil.

During the period of the experiment the alfalfa crop was harvested 29 times. The aggregate yield of dry matter for these crops from both cans was 5,510 gm. This mass of dry matter with reference to the

volume of water dissipated by evaporation and transpiration, shows that 0.772 liter was dissipated for each gram of dry matter produced. In other words, it required the dissipation of 772 gm. of water to produce 1 gm. of alfalfa dry matter. Another comparison that may be made involves the mass of salts absorbed or dissipated and dry matter of crop produced. If it is assumed that 744 gm. of salts was absorbed by and removed with the alfalfa crops, the mass of these salts is equivalent to 13.5 percent of the alfalfa dry matter.

In this experiment the observations were made on what may be taken as a small field of alfalfa, having a root zone approximately 18 inches deep and a surface area of 2.7 square feet. The record of water applied includes both the volume of irrigation water, containing known quantities of dissolved salts, and the volume of rain water, assumed to be free of dissolved salts. At the beginning of the period of record the two cans contained approximately 20 gm. of dissolved salts in the 40 liters of water held in the soil; at the end of the period they contained 44 gm. of dissolved salts. Thus the regimen of irrigation during the period was such that practically all the salt applied was removed from the root zone. In other words, with the input water having a mean salt concentration of 991 p. p. m. approximate salt balance was achieved when the volume of input was such that 22.5 percent of it passed through the root zone as percolate.

With this regimen of irrigation it was found that, of 5,443 gm. of salt added to the soil with the irrigation input, 4,675 gm., or 85.9 percent, was carried through the root zone by the percolating water. It is not possible to show definitely the destination of 768 gm. of salt that was not removed with the percolate. It seems probable that part of it, possibly the major part, was taken up by the alfalfa plants; but some of it may have been precipitated in or absorbed by the soil, or some of the constituents may have been decomposed and dissipated in other forms.

Data for 1938

During the first 3 years of the experiment the record did not include detailed analyses of the percolates; only the total dissolved salts were determined. For the period beginning with March 16, 1938, the observations included detailed analyses both of the input solutions and of the percolates. These detailed analyses included the determination of four cations—calcium, magnesium, sodium, and potassium—and four anions—bicarbonate (computed as carbonate), sulfate, chloride, and nitrate. Before the findings in respect to these several salt constituents are presented, the data for the 1938 period in respect to total salts will be compared with similar data for the whole period of record (table 8).

For the 1938 period (281 days) the volume of input for the two cans was 1,438.3 liters, containing 1,609.1 gm. of dissolved salts. This indicates a mean concentration of 1.119 gm. per liter. The volume of percolate was 389.4 liters, equivalent to 27.1 percent of the volume of input. This percolate contained 1,397.1 gm. of salt, or 86.8 percent of the input mass, and had a mean concentration of 3.588 gm. per liter. The volume of water dissipated was 1,048.9 liters and the mass of salt added but not recovered in the percolate was 212.0 gm., which includes 24 gm. additional residue in the soil. This mass of salts (188 gm. net), with reference to the volume of water dissipated in-

dicates a mean concentration of 0.179 gm. per liter. During this period six crops of alfalfa were harvested. They yielded 1,543 gm. of dry matter. This mass of dry matter, with reference to the volume of water dissipated, indicates a water requirement of 680 gm. of water for 1 gm. of dry matter. The mass of salt absorbed (188 gm.) is equivalent to 12.2 percent of the mass of alfalfa dry matter produced.

In comparing the data of the longer period with those of the included shorter period, it will be seen that the conditions and results were not greatly different. For the shorter period, the concentration of the input solution was slightly higher (1.119 : 0.991) and to achieve approximate salt balance for the root zone required a slightly higher ratio of percolate to input volnme (27.1 : 22. 5). The proportion of the mass of salts recovered in the percolate was also slightly higher (86.8 : 85.9). The water requirement, on the other hand, was slightly lower (680 as compared with 772), but the proportion of salt residue to alfalfa dry matter was 12.2 percent for the shorter period and 13.5 percent for the whole period.

It is appropriate at this point to describe the character of the salts used with the irrigation water of this experiment. Throughout the period of record Rubidoux tap water was used. During the period prior to March 16, 1938, the salinity of the irrigation water was increased, as desired, by adding to the tap water suitable quantities of sodium chloride. After March 16, 1938, calcium chloride was used instead of sodium chloride. The cans were not protected from the weather; consequently they were exposed to the natural rainfall, the volume of which to be credited to each can was computed from the volume collected in a standard Weather Bureau rain gage located near the cans. From time to time, as the needs of the alfalfa plants seemed to warrant, small quantities of salts containing nitrate, potash, and phosphate were added to the irrigation water. Each lot of irrigation water was sampled and analyzed [4] so that the record of input included the nitrate and potash added but not the phosphate.

TABLE 9.—*Record of experiment 28 from Mar. 16 to Dec. 21, 1938, showing the grams of each of 8 salt constituents added with the irrigation water* [1] *and collected with the percolate,*[2] *and the difference between these values*

Salt constituent	Input	Percolate	Difference
	Grams	*Grams*	*Grams*
Calcium (Ca)	476.0	345.8	130.2
Magnesium (Mg)	19.1	29.9	[3] 10.8
Sodium (Na)	77.5	129.5	[3] 52.0
Potassium (K)	14.9	3.5	11.4
Carbonate (CO_3) [4]	126.9	78.1	48.8
Sulfate (SO_4)	83.0	68.6	14.4
Chloride (Cl)	797.3	752.6	44.7
Nitrate (NO_3)	31.6	.4	31.2
Total	1,626.3	1,408.4	217.9

[1] Includes dissolved constituents in soil solution on Mar. 16.
[2] Includes dissolved constituents in soil solution on Dec. 21.
[3] Gain in output over input.
[4] Computed as half the bicarbonate (HCO_3).

The record of the salt constituents added to and collected from the soil of the two cans between March 16 and December 21, 1938, is shown in table 9. It will be noted at once that in respect to mag

[4] The writer is indebted to John T. Hatcher for making the analyses of the samples of irrigation water and percolate.

nesium and sodium the quantities found in the percolate exceeded the quantities added with the irrigation. For the six other constituents the quantities found in the percolate were less than those added with the irrigation. This recovery of more magnesium and sodium than was added is believed to be the result of reactions of base exchange that occurred in the soil. Prior to this period of the experiment the salts of the irrigation water had been chiefly sodium chloride, and it is assumed that sodium replaced some of the exchangeable calcium that the soil contained originally. The subsequent use of calcium chloride evidently caused the replacement of some of this sodium and of some magnesium as well.

It seems probable that the gains indicated for sodium and magnesium may not represent the full extent of the exchange reactions. It is to be assumed that these constituents may have been absorbed to some extent by the alfalfa plants. The quantities so absorbed should be added to the gains indicated to obtain the full values of the reactions of base exchange. In view of these gains of magnesium and sodium, it may be assumed that some part of the calcium unaccounted for (130.2 gm.) was involved in the reactions of base exchange. It is possible that the potassium may have been involved also, but in view of the low concentration of this constituent in the solution, it seems more probable that its chief destination was in absorption by the plants rather than in base-exchange reactions. With all of the anions, losses occurred. From the information available, it is not possible to estimate what proportion of these losses was due to plant absorption.

TABLE 10.—*Record of experiment 28 from Mar. 16 to Dec. 21, 1938, showing the gram equivalents of each of 8 salt constituents added with the irrigation water* [1] *and collected with the percolate,* [2] *and the difference between these values*

Salt constituent	Input (see table 9)	Percolate	Difference	Percolate
	Gram equivalents	*Gram equivalents*	*Gram equivalents*	*Percent* [3]
Cations:				
Calcium (Ca)	23.80	17.29	6.51	72.6
Magnesium (Mg)	1.57	2.46	[4] .89	156.7
Sodium (Na)	3.37	5.63	[4] 2.26	167.1
Potassium (K)	.38	.09	.29	23.7
Total	29.12	25.47	3.65	87.5
Anions:				
Bicarbonate (CO_3)	4.16	2.56	1.60	61.5
Sulfate (SO_4)	1.73	1.43	.30	82.7
Chloride (Cl)	22.46	21.20	1.26	94.4
Nitrate (NO_3)	.513	.006	.507	1.2
Total	28.863	25.206	3.667	87.3

[1] Includes dissolved constituents in soil solution on Mar. 16.
[2] Includes dissolved constituents in soil solution on Dec. 21.
[3] Percentage of input.
[4] Gain in output over input.

A clearer representation of the relationships of the salt constituents to one another can be obtained by expressing the values for input, output, and difference in gram equivalents rather than in grams. These relationships are shown in table 10. It will be observed that the values for input show that the sum of the cations exceeded slightly the sum of the anions. This may have been due in part to the

unavoidable discrepancies of analysis and in part to the omission from the computation of the small quantities of phosphate that were added with the irrigation water but not reported in the analysis. The values reported for the percolate show approximately the same discrepancy between the cations and anions. Here again there is uncertainty as to how much of the lower value for the sum of anions may have been due to errors of analysis and how much was due to greater losses or absorption of anions than of cations. It should be kept in mind that the values here reported represent the findings of 34 separate analyses.

The difference between the values for the input and for the percolate is shown both as gram equivalents and as percentage of the input value. The net loss for all cations (3.65) was 12.5 percent of the input; for the anions the corresponding percentage was 12.7. The largest percentage losses occurred with potassium and with nitrate. It is assumed that these losses were due largely to the absorption of these constituents by the alfalfa plants. In respect to the nitrate ion the values reported as milligram equivalents were as follows: Input 512.6, percolate 6.1, or a recovery of only 1.19 percent; but in reporting gram equivalents the nearest decimal values for two places were used. It is apparent that under the conditions of this experiment, which involved both a shallow root-zone and heavy leaching, there was not much loss by leaching of the two plant-food constituents.

The differences in the values for the cations show the most marked contrast. The percolate contained 3.15 gm. equiv. more of magnesium and sodium than was contained in the input solution. If it is assumed that these constituents were released from the soil by reactions of base exchange in which calcium was absorbed, then it appears that nearly half of the calcium that was lost was involved in these reactions. If allowance be made for the absorption of any magnesium and sodium by the alfalfa plants, then a corresponding quantity of additional calcium must be allocated to the exchange reactions. It may be repeated that during the period of the experiment prior to March 16, 1938, the salinity of the input solution had been due chiefly to sodium salt. This may explain why there was an abundance of replaceable sodium in the soil at the beginning of this last period of the experiment. However, it is not clear why so much magnesium was replaced from the soil.

For the four anions the mean loss, in gram equivalents, was 12.7 percent of the input values. The loss of nitrate was highest (98.8 percent). This is assumed to have been due largely to plant absorption. The next highest loss was in the carbonate constituent. Some of this loss may have been due to plant absorption, but it is possible that some of it was due to decomposition involving the absorption or dissipation of carbon dioxide and the precipitation of the carbonate as calcium carbonate in the soil. The percentage loss of sulfate was larger than the mean loss for all anions, but the actual quantity lost was small. On the other hand, the quantity of chloride lost was large but the percentage loss was small. It is assumed that most of the sulfate and chloride lost was absorbed by the alfalfa plants.

Each of the six alfalfa crops from each can for the 1938 period was analyzed for chloride and for total nitrogen. These analyses showed that the total crop (1,543 gm. of dry matter) contained 32.26 gm. of

nesium and sodium the quantities found in the percolate exceeded the quantities added with the irrigation. For the six other constituents the quantities found in the percolate were less than those added with the irrigation. This recovery of more magnesium and sodium than was added is believed to be the result of reactions of base exchange that occurred in the soil. Prior to this period of the experiment the salts of the irrigation water had been chiefly sodium chloride, and it is assumed that sodium replaced some of the exchangeable calcium that the soil contained originally. The subsequent use of calcium chloride evidently caused the replacement of some of this sodium and of some magnesium as well.

It seems probable that the gains indicated for sodium and magnesium may not represent the full extent of the exchange reactions. It is to be assumed that these constituents may have been absorbed to some extent by the alfalfa plants. The quantities so absorbed should be added to the gains indicated to obtain the full values of the reactions of base exchange. In view of these gains of magnesium and sodium, it may be assumed that some part of the calcium unaccounted for (130.2 gm.) was involved in the reactions of base exchange. It is possible that the potassium may have been involved also, but in view of the low concentration of this constituent in the solution, it seems more probable that its chief destination was in absorption by the plants rather than in base-exchange reactions. With all of the anions, losses occurred. From the information available, it is not possible to estimate what proportion of these losses was due to plant absorption.

TABLE 10.—*Record of experiment 28 from Mar. 16 to Dec. 21, 1938, showing the gram equivalents of each of 8 salt constituents added with the irrigation water [1] and collected with the percolate,[2] and the difference between these values*

Salt constituent	Input (see table 9)	Percolate	Difference	Percolate
	Gram equivalents	*Gram equivalents*	*Gram equivalents*	*Percent* [3]
Cations:				
Calcium (Ca)	23.80	17.29	6.51	72.6
Magnesium (Mg)	1.57	2.46	[4] .89	156.7
Sodium (Na)	3.37	5.63	[4] 2.26	167.1
Potassium (K)	.38	.09	.29	23.7
Total	29.12	25.47	3.65	87.5
Anions:				
Bicarbonate (CO_3)	4.16	2.56	1.60	61.5
Sulfate (SO_4)	1.73	1.43	.30	82.7
Chloride (Cl)	22.46	21.20	1.26	94.4
Nitrate (NO_3)	.513	.006	.507	1.2
Total	28.863	25.206	3.667	87.3

[1] Includes dissolved constituents in soil solution on Mar. 16.
[2] Includes dissolved constituents in soil solution on Dec. 21.
[3] Percentage of input.
[4] Gain in output over input.

A clearer representation of the relationships of the salt constituents to one another can be obtained by expressing the values for input, output, and difference in gram equivalents rather than in grams. These relationships are shown in table 10. It will be observed that the values for input show that the sum of the cations exceeded slightly the sum of the anions. This may have been due in part to the

unavoidable discrepancies of analysis and in part to the omission from the computation of the small quantities of phosphate that were added with the irrigation water but not reported in the analysis. The values reported for the percolate show approximately the same discrepancy between the cations and anions. Here again there is uncertainty as to how much of the lower value for the sum of anions may have been due to errors of analysis and how much was due to greater losses or absorption of anions than of cations. It should be kept in mind that the values here reported represent the findings of 34 separate analyses.

The difference between the values for the input and for the percolate is shown both as gram equivalents and as percentage of the input value. The net loss for all cations (3.65) was 12.5 percent of the input; for the anions the corresponding percentage was 12.7. The largest percentage losses occurred with potassium and with nitrate. It is assumed that these losses were due largely to the absorption of these constituents by the alfalfa plants. In respect to the nitrate ion the values reported as milligram equivalents were as follows: Input 512.6, percolate 6.1, or a recovery of only 1.19 percent; but in reporting gram equivalents the nearest decimal values for two places were used. It is apparent that under the conditions of this experiment, which involved both a shallow root-zone and heavy leaching, there was not much loss by leaching of the two plant-food constituents.

The differences in the values for the cations show the most marked contrast. The percolate contained 3.15 gm. equiv. more of magnesium and sodium than was contained in the input solution. If it is assumed that these constituents were released from the soil by reactions of base exchange in which calcium was absorbed, then it appears that nearly half of the calcium that was lost was involved in these reactions. If allowance be made for the absorption of any magnesium and sodium by the alfalfa plants, then a corresponding quantity of additional calcium must be allocated to the exchange reactions. It may be repeated that during the period of the experiment prior to March 16, 1938, the salinity of the input solution had been due chiefly to sodium salt. This may explain why there was an abundance of replaceable sodium in the soil at the beginning of this last period of the experiment. However, it is not clear why so much magnesium was replaced from the soil.

For the four anions the mean loss, in gram equivalents, was 12.7 percent of the input values. The loss of nitrate was highest (98.8 percent). This is assumed to have been due largely to plant absorption. The next highest loss was in the carbonate constituent. Some of this loss may have been due to plant absorption, but it is possible that some of it was due to decomposition involving the absorption or dissipation of carbon dioxide and the precipitation of the carbonate as calcium carbonate in the soil. The percentage loss of sulfate was larger than the mean loss for all anions, but the actual quantity lost was small. On the other hand, the quantity of chloride lost was large but the percentage loss was small. It is assumed that most of the sulfate and chloride lost was absorbed by the alfalfa plants.

Each of the six alfalfa crops from each can for the 1938 period was analyzed for chloride and for total nitrogen. These analyses showed that the total crop (1,543 gm. of dry matter) contained 32.26 gm. o

chloride and 42.19 gm. of nitrogen. The data of table 9 show that the chloride content of the input (irrigation water) was 797.3 gm. and that the output (percolate) contained 752.6 gm. If the chloride found in the alfalfa be added to that contained in the percolate (752.6+32.26 gm.), the sum is 784.86 gm. This sum subtracted from the input chloride (797.3−784.86 gm.) leaves a difference of only 12.44 gm. of chloride, or 1.56 percent of the input chloride, unaccounted for during the period of the experiment.

The data as to nitrogen show a situation strikingly different from that of the chloride. For the 1938 period of the experiment the input of nitrate nitrogen (512.6 mg. equiv.) was 7.18 gm. The input solution may have contained some nitrogen in other forms, e. g., as ammonium salts, but the quantity was probably small. The percolate for the same period contained only 6.1 mg. equiv. of nitrate, equivalent to 0.085 gm. of nitrogen. On the other hand the crop contained 42.19 gm. of nitrogen. Thus the total output of nitrogen, in crop and percolate, was 42.275 gm. of nitrogen whereas the input was 7.18 gm. This indicates that the crop absorbed from the air and soil, through biological processes, approximately 35 gm. of nitrogen. This would be equivalent to 45 pounds of nitrogen for a ton of dry alfalfa.

DISCUSSION AND SUMMARY

FIELD INVESTIGATIONS

Results of the field investigations afford information on (1) the volume of stream water annually entering or delivered to a large irrigated area, together with the quantity and composition of the dissolved salts contained in the water, and (2) the volume of water annually leaving the same area as stream flow or drainage, together with the quantity and composition of the dissolved salts contained in that water. With optimum conditions it should be possible to ascertain from these data information on (1) the volume of water annually dissipated from the area as evaporation and transpiration, by taking the difference between the inflow and the outflow, and (2) the quantity and composition of the dissolved salts annually deposited in or removed from the soil of the area, by taking the differences in quantity and composition between the incoming and outgoing salts.

Under field conditions it is not practicable to determine accurately, by difference, the volume of water annually dissipated by evaporation and transpiration, because the actual input of water includes not only the stream flow but also rain water. The volume of annual precipitation falling within the boundaries of the irrigated areas might be estimated fairly accurately, but each of these areas is surrounded by higher ground from which there may be some run-off of storm water or some movement of ground water into the irrigated area. Therefore it is difficult to arrive at an acceptable value for total volume of input and consequently to compute the volume of dissipated water by subtracting the measured output.

In respect to the salt balance, the situation is different. The quantities of dissolved salt carried into and removed from the irrigated area by the stream waters and drainage may be computed witb acceptable accuracy. And it may be assumed that the quantity of salt contributed directly or indirectly by the precipitation is so small as to be negligible. Thus, the quantity of salt brought in by the stream water

or irrigation supply may be taken as total input. The total salt output, however, includes not only the dissolved salts carried away from the area by the drainage and unused stream water, i. e., the total measured outflow, but also (1) the quantity of dissolved salts that may have been absorbed by and removed with the crops, (2) the quantity that may have been precipitated from solution in the soil, and (3) the quantity of such salt constituents as may have been decomposed in the soil, e. g., bicarbonate or nitrate, or may have been involved in reactions of base exchange, e. g., calcium, magnesium, and sodium. Therefore, the net salt balance, as computed by taking the difference between the quantities carried by stream or irrigation input and stream or drainage output, may be in error by the amount of the quantities absorbed, precipitated, or decomposed.

In the present consideration of these areas no attempt has been made to determine what proportion of the input salt passed into and remained in the subsoil water of the area, nor to estimate what proportion of the output salt originated from root-zone leaching as a direct result of irrigation and what proportion was contributed from subsoil water originally present in the area. The data presented relate to each area as a whole, including not only the root zone of the soil but also the deeper water-saturated sediments. It is conceivable that with a very large reservoir of subsoil water in an area and with conditions such that a large part of the input salt passes into that reservoir, an adverse salt balance may continue for a long time without involving the accumulation of harmful quantities of salts in the solution of the root zone.

On the other hand, conditions within the area may be such that a large proportion of the incoming irrigation water passes directly into the subsoil reservoir by way of percolation losses from canals. In that event the outflowing drainage water may represent largely water displaced from the subsoil reservoir and containing dissolved salts in high concentration and of remote origin. Under such conditions there may long continue for the area as a whole a favorable salt balance, i. e., output exceeding input, and yet with inadequate root-zone leaching there may be progressive and harmful accumulation of salts in the root zone.

From these considerations it follows that field observations as to salt-balance conditions cannot be expected to yield precise information as to the volume of water annually dissipated by evaporation and transpiration unless supplemented by precise information as to the rainfall contributions. It follows, also, that because of inherent uncertainty as to the volume, concentration, and composition of the original ground water in an irrigated area and as to the extent of its contribution to the drainage outflow, the findings of a short-period salt-balance investigation should not be interpreted as applicable to the surface soil of that area. These findings must be considered as applying to the whole system, which includes the surface or crop-producing horizon of the soil together with the underlying ground water. Field observations extending over a few years should show the trend of events in respect to salt balance and should indicate whether existing drainage facilities are adequate to maintain the present equilibrium for the area as a whole. It is manifestly desirable to know the facts in respect to the trend of change in salinity conditions

The conditions of salinity that directly affect crop production are those that exist in the surface soil or root zone. It is chiefly the water supplied to the root zone by irrigation or rainfall that is used by plants. If this water contains dissolved salt constituents in excessive concentrations, crop growth is impaired. It is therefore appropriate to obtain information concerning salt-balance conditions in the root zone as affected by the character and the quantity of the irrigation water. Such information is not readily obtainable from field investigations. More precise information may be obtained by growing crop plants in soil under conditions that permit the accurate measurement of the volume of water applied and the volume of water that percolates through the root zone. It is necessary also to determine the concentration and composition of the dissolved salts in the irrigation water and in the drainage water.

LABORATORY EXPERIMENT

The objective of the laboratory experiment here reported was to obtain information as to salt-balance relationships to supplement the information currently obtained by observation of conditions in irrigated areas. Particularly it was desired to ascertain (1) what proportion of the irrigation water must be allocated to root-zone leaching in order to prevent the accumulation of soluble salts in the root zone and (2) what proportion of the dissolved salts of the irrigation supply is absorbed by the crop plants.

The results of the experiment show that for the whole period of 4½ years, during which the mean salt concentration of the irrigation water was 991 p. p. m. (1.35 tons per acre-foot), it was necessary to allocate 22.5 percent of the input to root-zone leaching in order to prevent salt accumulation in the soil. For the included shorter period of 9 months, during which the mean concentration of the irrigation water was 1,119 p. p. m. (1.52 tons per acre-foot), the necessary allocation to root-zone leaching was 27.1 percent of the irrigation input.

In respect to the second question, viz, the proportion of the dissolved salts that was absorbed by the crop plants (alfalfa), or by the soil, the findings for the longer period show that 13.7 percent of the total salts contained in the input water was so absorbed. During the shorter and final period the proportion so absorbed was 11.7 percent of the input.

The volume of water dissipated by evaporation and transpiration during the whole period of the experiment weighed 772 times as much as the dry weight of the alfalfa produced. For the included and final period of the experiment, the volume of water dissipated weighed 680 times as much as the dry weight of the alfalfa crops. The mass of dissolved salts absorbed by the crop plants and by the soil, i. e., the quantity in the input minus the quantity in percolate was, for the whole period, equivalent to 13.7 percent of the dry weight of the crop produced.

SUMMARY

The relation between the quantity of dissolved salts delivered to an irrigated area with the irrigation water and the quantity removed from the area by the drainage water is described as the salt balance of

he area. A favorable salt balance exists when the output of salts equals or exceeds the input. The salt balance is adverse when the nput exceeds the output.

The conditions of irrigation and of drainage are described for three rrigated areas, two on the Rio Grande in New Mexico and Texas and one on the Colorado River in Arizona.

The methods of determining and computing the quantities of salts entering and leaving an irrigated area are described, and the salt-balance conditions for each area for a period of years are reported.

The data for these irrigated areas include also the relative input and output of each of the seven more important salt constituents and thus show the changes in the composition of the dissolved salts that occur in each area.

The field observations are supplemented by the data of a laboratory experiment that had two major objectives, namely, with a given concentration of dissolved salts in the irrigation water, to determine (1) what proportion of the irrigation input must be allocated to root-zone leaching in order to prevent the accumulation of soluble salts in the soil of the root zone and (2) what proportion of the dissolved salts contained in the irrigation water is removed from solution by the crop plant (alfalfa) or by the soil.

BORON ABSORPTION BY SUNFLOWER SEEDLINGS[1]

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INTRODUCTION

It has come to be generally accepted as a fact that many, if not all, crop plants require boron in order to make normal growth. In respect to a number of crop plants, definite and characteristic symptoms have been identified as due to a deficiency of available boron in the soil. The literature in this field has been abstracted and several comprehensive bibliographies prepared (*3*, *4*).[2 3] It was shown by Mazé (*7*) and later confirmed by Warington (*11*) that boron must be included among the essential constituents of artificial solutions for the production of normal plants in water cultures. Boron is thus one of an indeterminate number of elements (*14*), such as iron, manganese, and zinc, that are essential to normal plant growth. Since the essential requirements of plants for these elements may be served by relatively small quantities as compared with the required quantities of such elements as carbon, nitrogen, potassium, and phosphorus, the term "miotrophic"[4] is proposed for this group. In respect to plant nutrition, the miotrophic group includes such elements as boron, iron, manganese, and zinc, which, though essential, are required in very small quantities.

Although boron is an essential constituent of the soil solution for normal plant growth, it is injurious to most plants when its concentration in the soil solution exceeds a few parts per million. Crop injuries have been reported as resulting from the use of commercial fertilizers containing salts of boron (*2*, *8*, *10*) and also from the use in irrigation of natural waters containing boron (*6*). In previous publications (*5*, *9*) it has been shown (*a*) that solution concentrations of boron above 3 or 4 p. p. m. may be injurious to many crop plants and (*b*) that the boron content of the plant material, particularly of the leaves, increases with the concentration of boron in the supporting solution.

In making field surveys to find and delimit the sources of boron that occur in certain irrigation waters of the Western States, it was found that the work could be expedited by collecting and analyzing the leaves of certain widely distributed plants. For example, the leaves of such trees as the walnut and any of the common species of citrus show, by characteristic markings and by analysis, whether supernormal concentrations of boron are present in the soil solution. Similarly, a number of herbaceous plants, including the sunflower and alfalfa, contain supernormal quantities of boron if the boron concentration of the soil solution is abnormally high. In view of these

[1] Received for publication May 1, 1940.

[2] AMERICAN POTASH INSTITUTE. BORON AS A PLANT NUTRIENT. A BIBLIOGRAPHY OF LITERATURE PUBLISHED AND REVIEWED JULY 1938 TO DECEMBER 1938, INCLUSIVE. Sup 1, 40 pp. Washington, D. C., 1939. [Mimeographed.]

[3] Italic numbers in parentheses refer to Literature Cited, p. 56.

[4] A new word from Greek *mio-* (lesser) and *trophikos* (fr. *trophos*, feeder).

findings, it seems appropriate to explore the possibilities of the indicator-plant method in verifying and delimiting areas of boron deficiency.

In the course of investigating the subject of boron toxicity, a method of analysis (*1*, *12*) has been developed by which it is possible to measure, with a fair degree of precision, quantities of boron as small as 0.01 mg. in aqueous solution. By an adaptation (*3*) of this method it is possible to determine accurately the quantity of boron in plant material. It is possible also to determine, with acceptable accuracy, the total quantity of boron in a sample of soil, and the quantities of water-soluble and of acid-soluble boron in soils. There remains, however, some doubt as to a reliable method of determining, in a soil sample, the quantity of boron that is available to plants. This uncertainty has made it necessary to obtain information as to the relationship between the concentration of boron in a nutrient solution and the quantity of boron contained in plants grown therein.

SUNFLOWERS GROWN IN SOLUTION CULTURES

The sunflower was used in this investigation because (1) the plant grows well under a wide range of natural and artificial conditions, (2) it produces in a few weeks' time a relatively large quantity of plant material for analysis, and (3) it tolerates a wide range of boron concentrations. The first objective was to determine whether clear-cut symptoms of boron deficiency could be produced in sunflower seedlings by growing the plants in a nutrient solution made with ordinary laboratory reagents that were known to contain traces of boron.

EFFECT OF BORON ON GROWTH OF PLANTS

Experiment 1

Experiment 1 included three varieties of sunflowers and two culture solutions. The sunflower varieties were Mammoth Russian, California Double, and California Native. The first culture solution contained the following salts (millimoles per liter): Calcium nitrate ($Ca(NO_3)_2$), 1.25; potassium nitrate (KNO_3), 1.25; magnesium sulfate ($MgSO_4$), 0.50; sodium chloride ($NaCl$), 0.50; and monopotassium phosphate (KH_2PO_4), 0.25; together with very small quantities of the sulfates of iron, manganese, and zinc. The second culture solution differed from the first by having added to it a quantity of boric acid to give it a boron concentration of 2.5 p. p. m. Several analyses of the culture solutions to which no boron was added showed that it contained approximately 0.01 p. p. m. of boron.

The sunflower seedlings, shortly after germination, were set in the perforated tops of 1-liter glass jars, with three or four plants to each jar. During the 40 days after the seedlings were set into jars the solutions were restored to volume daily with distilled water, and four times during that period the used solutions were discarded and replaced with fresh solutions. The plants were set in the solutions in the greenhouse at the Rubidoux Laboratory, Riverside, Calif., January 30, 1939. By February 10 it became apparent that the plants grown in the solutions to which no boron had been added were not growing so well as the others. The difference in growth rate became greater as time went on. The plants grown without added boron made very little growth beyond the formation of the first leaves. The first inter-

node above the cotyledons was somewhat shortened, and the growing point soon died without producing the second internode.

The comparative growth made by plants of the three varieties of sunflowers in 6 weeks in the culture solution is shown in figure 1 and in table 1. It is obvious from figure 1 that the quantity of boron contained as impurities in the reagent salts used for the culture solution was insufficient to support normal growth of the seedlings. The

FIGURE 1.—Sunflower seedlings grown for 6 weeks in culture solutions with (*a*) and without (*b*) added boron: *A*, Mammoth Russian; *B*, California Double; *C*, California Native.

weights of dry matter reported in table 1 show that the plants without added boron made only 15 to 25 percent as much growth as those having an ample supply of boron.

TABLE 1.—*Dry weight, boron content, and boron concentration of each lot of sunflower seedlings of experiment 1*

Culture solution and variety	Plants	Leaves and stems		
		Dry material	Boron in dry material	
	Number	*Grams*	*Milligrams*	*Parts per million*
No boron added:				
Mammoth Russian	20	8.1	0.12	15
California Double	16	4.1	.05	12
California Native	6	2.1	.04	19
Boron 2.5 p. p. m.:				
Mammoth Russian	20	45.6	15.45	339
California Double	16	27.4	8.66	316
California Native	7	8.4	2.38	283

The data of table 1 show the number of plants in each group, the weight of dry material in the leaves and stems, the quantity of boron

weight. In respect to the concentration of boron in the plant tops, the differences resulting from the two solutions are very large. The mean concentration of the plants grown without boron was 15 p. p. m. while those having an ample supply of boron had a mean concentration of 313 p. p. m., or 20 times as much. The results of this experiment showed that sunflower seedlings require for normal growth more boron than occurs as impurities in the ordinary reagent salts used in making culture solutions. They showed also that the plant material grown with an ample supply of boron contained much more boron than that grown with a deficient supply.

Experiment 2

For experiment 2, only 1 variety of sunflower, the Mammoth Russian, was used. The same type of equipment was employed as in experiment 1. Each group consisted of 24 plants in 6 jars of 1-liter capacity. The solutions used were the same as in the first experiment, except that one more was used containing boric acid equivalent to 0.1 mg. of boron per liter of solution. Again, the seedlings were grown for 6 weeks in 5 successive solutions. The seedlings were placed in the solutions on February 24, 1939. The comparative growth made by the plants in the 3 boron concentrations is shown in table 2 and in figure 2 from a photograph made on April 4, 1939, after 39 days.

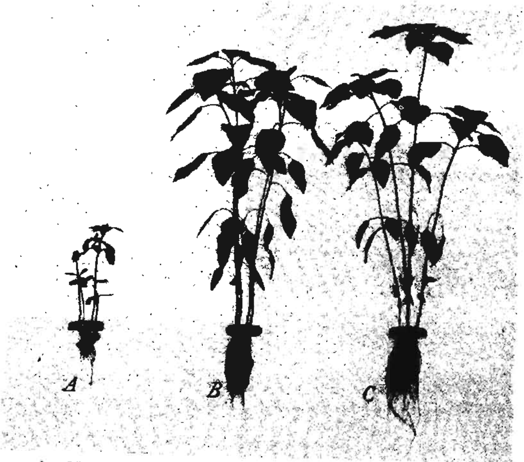

Figure 2.—Mammoth Russian sunflower seedlings grown for 6 weeks in culture solutions containing: *A*, No added boron; *B*, 0.1 p. p. m.; and *C*, 2.5 p. p. m. of added boron.

TABLE 2.—*Dry weight, boron content, and boron concentration of each lot of Mammoth Russian sunflower seedlings of experiments 2 to 4*

Experiment No.	Plants	Boron added to culture solution	Leaves and stems		
			Dry material	Boron in dry material	
	Number	*Parts per million*	*Grams*	*Milligrams*	*Parts per million*
2	24	0.0	7.6	0.11	14
	24	.1	56.5	2.18	39
	24	2.5	51.6	11.84	229
3	16	0	2.5	.03	12
	16	.025	24.5	.68	28
	16	.05	33.3	.99	30
	16	.075	35.0	1.23	35
	16	.1	36.3	1.36	37
4	16	0	1.5	.02	13
	16	.025	37.0	.89	24
	16	.05	45.4	1.37	30
	16	.075	46.0	1.85	40
	16	.1	43.4	2.07	48

The data of table 2 confirm the evidence obtained in the first experiment in respect to the comparable groups of plants. The growth made in solutions with no added boron was very restricted. On the other hand, the growth made in solutions containing 0.1 p. p. m. of boron was slightly larger than that made in the solution containing 2.5 p. p. m. The plants grown in the boron-deficient solution contained 0.11 mg. of boron, equivalent to 14 p. p. m. with reference to the dry material. The plants grown in the solution containing 0.1 p. p. m. of boron contained 2.18 mg. of that element. The 30 liters of solution to which these plants had access contained approximately 3.3 mg. of boron. This indicates that these plants absorbed about two-thirds of the boron available to them. The results of this experiment suggest that with sunflower seedlings the essential concentration of boron is less than 0.1 p. p. m. in the solution.

EXPERIMENT 3

The objective of experiment 3 was to explore the effects of boron concentrations of 0.1 p. p. m. and less. The same type of equipment was used as in experiments 1 and 2. Each group consisted of 16 plants in 4 jars. The same type of solution was used with boron added in concentrations of 0.025, 0.05, 0.075, and 0.1 p. p. m. There were successive solutions used during the 6 weeks of the experiment, the volume being restored daily with distilled water. The sunflower seedlings, Mammoth Russian, were set in the solutions on April 8, 1939. The comparative growth made in each solution is shown in table 2 and in figure 3, *A–E*, from a photograph made on May 19, 1939.

Table 2 (experiment 3) and figure 3, *A–E*, show that even 0.025 mg. of boron per liter added to the culture solution made possible greatly increased growth in the sunflower seedlings as compared with growth in culture solutions to which no boron was added. The next increment of boron (0.05 p. p. m.) caused a further increase of growth of 36 percent. The increased growth in the next two solutions was not very great. The plants in the solutions without added

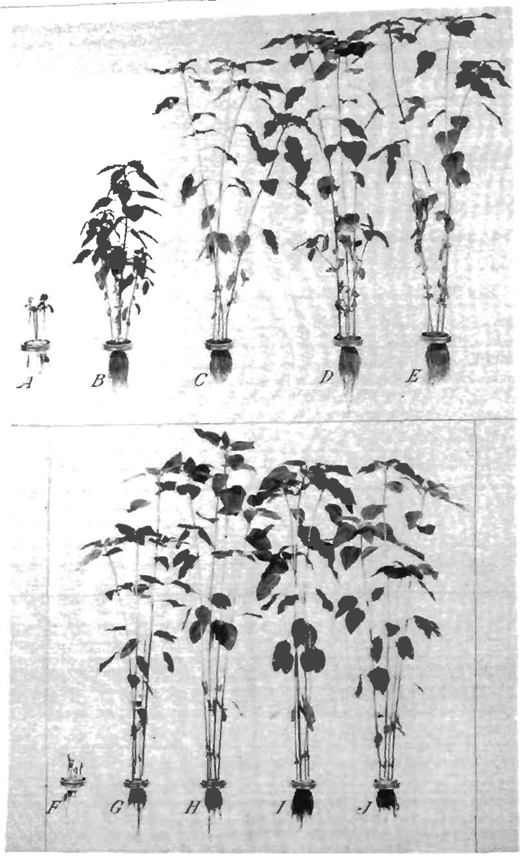

FIGURE 3.—Sunflower seedlings grown for 6 weeks in culture solutions containing: *A*, No added boron; *B*, 0.025 p. p. m.; *C*, 0.05 p. p. m.; *D*, 0.075 p. p. m.; and *E*, 0.1 p. p. m. of added boron; *F*, No added boron; *G*, 0.025 p. p. m.; *H*, 0.05 p. p. m.; *I*, 0.075 p. p. m.; and *J*, 0.1 p. p. m. of added boron.

boron made almost no growth beyond the cotyledon stage. Each group of plants had access to the boron contained in 16 liters of culture solution, 4 jars each, and 4 successive solutions. Thus the quantity in milligrams of boron available to each group of plants was, respectively, 0.16, 0.56, 0.96, 1.36, and 1.76, including the boron presumably contributed by the chemicals used in making up the solutions. It may be noted that the 16 sunflower seeds used for each group of plants contained approximately 0.025 mg. of boron.

The quantities of boron found in the plants of two of the groups exceeded slightly the quantities estimated as available in the solutions. Slight discrepancies in these relative values are not surprising because the quantities involved are very small and approach the limits of accuracy of the determinations. It is evident from the data reported that in this experiment the plants took from the solutions practically all the boron that was present. The concentrations of boron in the dry plant material increased consistently with the increases in the culture solutions, and concentrations in experiment 3 are very close to those shown for corresponding solutions in experiment 2 (table 2).

Experiment 4

Experiment 4 was essentially a repetition of experiment 3. It differed in that it was conducted later in the season, May 13 to July 25, when the temperatures were higher, and also in that the culture solutions were renewed more frequently. Otherwise, the method and the solutions used were the same as in experiment 3. With the advance of the season the seedlings grew faster and transpired more water. Observations as to the concentrations of the used solutions in experiment 3 had shown that the nutrient constituents were exhausted, and it was thought that more frequent solution renewals would give better results. In the 6 weeks' duration of experiment 4 there were nine successive solutions used as compared with four successive solutions in experiment 3.

The more frequent change of solutions increased not only the supply of nutrient constituents available to the plants but also the quantity of boron. The 9 successive solutions used made available to each group of 16 plants the following quantities of boron (in milligrams): 0.36, 1.26, 2.16, 3.06, and 3.96, including the boron presumably contained in the reagents used for the nutrient solutions. The comparative growth of the sunflower plants during the 6 weeks of the experiment is shown in figure 3, *F–J*, and in table 2.

The data of table 2 show that in experiment 4, as in experiment 3, there was a striking increase in growth as between the plants in the solution containing no added boron and those in the solution to which so small a quantity as 0.025 mg. per liter was added. In fact, most of the plants without added boron died before the experiment was concluded. The plants receiving 0.05 p. p. m. of boron made only 23 percent more growth than those receiving 0.025 p. p. m., and there was no increased growth with the solutions of higher boron concentration. It seems probable that the more frequent renewal of the solutions in this experiment supplied enough more boron to account in part for the growth differences shown in the two experiments.

The ratios of quantities of boron found in the plants to the quantities available in the solutions were as follows: 0.025 p. p. m., 71 percent;

0.05 p. p. m., 69 percent; 0. 075 p. p. m., 60 percent; and 0.1 p. p. m., 52 percent. However, the boron concentrations of the plant material increased progressively with increases of boron in the culture solutions.

WATER ABSORBED AND TRANSPIRED

In the course of experiments 2, 3, and 4, a record was made of the quantities of distilled water added daily to each jar of culture solution to restore the volume. These records give a measure of the quantities of water absorbed and transpired by each group of plants. Experience has shown that with a given plant and similar climatic conditions the quantity of water transpired is closely related to the quantity of plant material produced, and the term "water requirement" is used for the ratio between the two quantities. In table 3 are shown the volumes of water lost by each group of plants grown in experiments 2, 3, and 4. No record was made of the water used in experiment 1. The table also shows the weights of dry material produced by each group of plants and the water requirement for each group. It will be noted that the water requirement increased with the advance of the season from March to July.

TABLE 3.—*Volumes of water transpired by sunflower plants of experiments 2, 3, and 4, and water requirement of each group of plants*

Experiment No.	Plants	Boron in solution	Water transpired	Dry material of leaves and stems	Water requirement
	Number	*Parts per million*	*Liters*	*Grams*	*Liters÷grams*
2	24	0.01	4.37	7.6	575
		.1	29.29	56.5	518
		2.5	26.08	51.6	505
3	16	.01	1.42	2.5	568
		.025	16.29	24.5	665
		.05	18.94	33.3	569
		.075	20.10	35.0	574
		.1	20.84	36.3	574
4	16	.01	1.21	1.5	807
		.025	27.54	37.0	744
		.05	33.81	45.4	745
		.075	36.81	46.0	800
		.1	33.71	43.4	777

The record of the volumes of water absorbed and transpired confirms other evidence, viz, that of the apperance of the plants and of the quantity of plant material produced, in showing that boron deficiency sharply curtailed plant growth. In general, the water requirement was slightly higher with the boron-deficient cultures but the differences were not great.

In these experiments the volume of the culture solutions was maintained by filling the jars from day to day with distilled water, and each culture solution, as restored to volume, was analyzed after it had been replaced by a new solution. These analyses show that the used solutions were of much lower concentration than the new solutions; in other words, that the plants had absorbed the dissolved constituents along with the water. Most of the water absorbed was subsequently transpired, but the dissolved constituents were retained by the plants. A record of the volume of water taken up by each group of plants and

a record of the boron found in each group make it possible to show, by the ratios between these values, the computed boron concentration of the water taken up by the plants. Table 4 shows the two values for each group of plants and the corresponding ratios.

TABLE 4.—*Volumes of water taken up by Mammoth Russian sunflower plants, boron content of the plants, and ratios of the two values*

Boron in solution (parts per million)	Experiment	Water taken up	Boron in leaves and stems	Ratio of boron to water taken up
	No.	*Liters*	*Milligrams*	*Parts per million*
0.01 [1]	2	4.37	0.11	0.025
	3	1.42	.03	.021
	4	1.21	.02	.016
0.025	3	16.29	.68	.042
	4	27.54	89	.032
0.05	3	18.94	.99	.052
	4	33.81	1.37	.041
0.075	3	20.10	1.23	.061
	4	36.81	1.85	.050
0.1	2	29.29	2.18	.074
	3	20.84	1.36	.065
	4	33.71	2.07	.061
2.5	2	26.08	11.84	.454

[1] No boron added.

These data permit comparison between the determined boron concentration of the culture solution and the computed concentration of the water taken up. For the plant groups having culture-solution concentrations below 0.05 p. p. m. of boron, the boron concentration of the water taken up by the plants was higher than that of the culture solution. For the plant groups having solution concentrations above 0.05 p. p. m. of boron, the boron concentration of the water taken up by the plants was lower than that of the culture solution. In other words, when the supply of boron in the culture solution was such as to provide 0.05 mg. of boron for each liter of water absorbed by the plants, the normal processes of growth could go on. The inference that seems warranted is that with sunflower seedlings, symptoms of boron deficiency occur when the boron concentration of the available solution is less than 0.05 p. p. m.

PLANT WATER

The sunflower seedlings of these experiments contained a high proportion of water. When an experiment was concluded after approximately 6 weeks, the plants were removed from the culture jars, the stems were cut at the junction with the roots, and the leaves were detached from the stems. The green weights were then recorded for the roots, the stems, and the leaves of each group of plants except for the plants grown in the solutions without added boron. The plants of these cultures did not produce normal leaves, so the upper part of the plant with the cotyledons and partially formed leaves was classed as stems. After the green weights had been recorded, the plant material was dried to constant weight at 70° C. The loss in weight on drying is here designated as plant water.

Because the water contained in the living plant is in contact with that of the supporting solution and because the proportion of water to dry matter may be very different in the roots, stems, and leaves, the authors feel warranted in reporting the relationship between the volume of plant water and the boron found in the plant material. In calling attention to this relationship, it is not implied that the boron is all held in solution in the plant water. Some of it, particularly in the leaves, is probably combined with the plant material and is not in solution. However, the plant water constitutes a system that is interconnected throughout the plant and may serve as a common denominator to show which parts of the plant contain relatively the most boron. Table 5 shows the plant water, the plant boron, and the ratios between these values for the roots, stems, and leaves of the sunflowers grown in the experiments here reported.

TABLE 5.—*Volume of plant water, boron content, and computed boron concentration of plant water in the roots, stems, and leaves of Mammoth Russian sunflower seedlings*

Boron in culture solution (parts per million)	Experiment	Plant water	Boron	Computed boron concentration of plant water	Mean
ROOTS					
	No.	*Milliliters*	*Milligrams*	*Parts per million*	*Parts per million*
0.01[1]	1	33.2	0.14	4.2	2.2
	2	26.1	.06	2.3	
	3	10.1	.01	1.0	
	4	8.1	.01	1.2	
0.025	3	79.1	.27	3.4	2.9
	4	92.2	.22	2.4	
0.05	3	90.2	.30	3.3	3.0
	4	107.3	.29	2.7	
0.075	3	108.0	.30	2.8	2.3
	4	132.6	.25	1.9	
0.1	2	230.3	.47	2.0	2.5
	3	118.9	.37	3.1	
	4	123.8	.28	2.3	
2.5	1	253.1	.88	3.5	4.0
	2	198.3	.90	4.5	
STEMS					
0.01[1]	1	64.9	[2]0.12	1.8	1.9
	2	71.0	[2].11	1.5	
	3	18.5	[2].03	1.6	
	4	7.6	[2].02	2.6	
0.025	3	94.3	.20	2.1	2.2
	4	199.8	.48	2.4	
0.05	3	148.1	.33	2.2	2.1
	4	287.1	.55	1.9	
0.075	3	149.3	.36	2.4	2.1
	4	290.0	.53	1.8	
0.1	2	278.3	.54	1.9	2.1
	3	165.8	.36	2.2	
	4	255.0	.54	2.1	
2.5	1	232.5	.82	3.5	4.1
	2	158.4	.74	4.7	

[1] No boron added.
[2] Stems with cotyledons and stunted leaves.

TABLE 5.—*Volume of plant water, boron content, and computed boron concentration of plant water in the roots, stems, and leaves of Mammoth Russian sunflower seedlings*—Continued

LEAVES

Boron in culture solution (parts per million)	Experiment	Plant water	Boron	Computed boron concentration of plant water	Mean
	No.	*Milliliters*	*Milligrams*	*Parts per million*	*Parts per million*
0.01 [1,3]					
0.025	3	92.0	0.48	5.2	4.5
	4	107.1	.41	3.8	
0.05	3	89.3	.66	7.4	7.1
	4	121.1	.82	6.8	
0.075	3	92.1	.87	9.4	9.8
	4	129.9	1.32	10.2	
0.1	2	178.2	1.64	9.2	10.9
	3	86.0	1.00	11.5	
	4	128.3	1.53	11.9	
2.5	1	141.9	14.63	103.0	86.4
	2	159.0	11.10	69.8	

[1] No boron added.
[3] These plants produced no normal leaves.

The data of table 5 show that in the roots the ratio of boron to plant water is low, i. e., 2 to 4 p. p. m. and that the difference is not great as between the supporting solution containing 0.025 p. p. m. and the one containing 100 times as much. In the latter case the boron concentration of the plant water is only slightly higher than that of the culture solution.

It appears also that in the plant stems the ratio of boron to plant water is approximately the same as in the roots. This suggests that in the absorbing and transporting tissues of the plant normal growth processes may go on if the boron content of the plant water of these tissues is equivalent to 2 p. p. m.

In the leaves the boron ratios are higher. Furthermore, with the leaves these ratio values increase progressively with the increase of available boron in the culture solution. This may imply that in the photosynthetic tissues more of the boron is necessarily withdrawn from the plant-water system.

BORON CONTENT OF DRY PLANT MATERIAL

One of the objectives of the investigations here reported was to determine the suitability of the sunflower as a plant to be used as an indicator in verifying or delimiting areas of boron deficiency in the soil. The need in such survey work is a plant that grows rapidly and under a wide variety of conditions, either in natural soils or in soil samples, and particularly a plant that absorbs boron in proportion to the quantity of that element available in the soil solution. Because it is usually more convenient to collect and use for analysis the dry plant material, the appropriate data from these sunflower experiments have been assembled in table 6 to show the boron content of the roots, the stems, and the leaves.

These data show the boron content of the dry material expressed as parts per million. Consideration of the boron content of the root

material is less pertinent in this connection because it is not practicable to collect the roots of plants grown in the soil. The boron content of the stem material does not increase measurably with slight increases in the boron content of the culture solution. On the other hand, the boron content of the leaves increases progressively with the increase in boron in the solution. Through the range of solution concentrations from 0.025 to 0.075 p. p. m. of boron, the boron content of the leaves increases by 16 p. p. m. with an increase of 0.025 p. p. m. in the boron content of the solution. It is not to be expected that exactly this relationship would be found to occur generally. But it may be expected that if sunflower leaves contain less than 50 p. p. m. of boron this may be taken as an indication of boron deficiency.

TABLE 6.—*Boron content of dry material of roots, stems, and leaves of Mammoth Russian sunflowers grown in solutions having different proportions of available boron*

Boron in culture solution (parts per million)	Experiment	Boron in dry material of— Roots	Stems	Mean	Leaves	Mean
	No.	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*
0.01 [1]	1	76	[2] 15			
	2	48	[2] 14			
	3	12	[2] 12			
	4	22	[2] 14			
0.025	3	61	20	22	33	28
	4	34	24		24	
0.05	3	51	18	19	45	44
	4	40	21		43	
0.075	3	45	18	19	57	60
	4	30	21		64	
0.1	2	34	17	19	66	70
	3	54	17		66	
	4	36	23		77	
2.5	1	63	32	30	728	586
	2	77	28		444	

[1] No boron added. [2] Includes cotyledons and stunted leaves.

These findings in respect to the boron content of the plant material make it clear that it is not necessary or even desirable in boron-survey investigations to collect and analyze the whole sunflower plant. Even with seedling plants only 6 weeks old, the dry weight of the stems usually exceeds that of the leaves. Because of the fact that the stem material has relatively a low and constant boron content, the inclusion of stem material with the leaf material in samples taken for analysis would tend to diminish the differences that are sought.

SUNFLOWERS GROWN IN A SAND CULTURE

In addition to the solution-culture experiments here reported, another experiment (No. 5) was conducted. In this experiment the plants were grown in sand and watered with solutions similar to those used in experiment 1, viz, a solution to which no boron was added and a solution containing 2.5 p. p. m. of boron. The sand-culture equipment consisted of 12 Oldberg percolators, each containing approximately 2 kg. of sand and 4 sunflower plants of the Mammoth Russian variety. The solutions were applied to the sand in excess. The percolates were collected, restored to volume with distilled water, and re-used for several days. Five successive fresh

The sand used in this experiment had been used previously in experiments dealing with boron toxicity, and consequently it had been in contact with solutions containing relatively high concentrations of boron. Before its use in this sunflower experiment the sand had been washed carefully, both with acid solutions and with distilled water, so that its boron content must have been very low. The sunflower plants grew very well in these sand cultures. Their appearance 6 weeks after germination is shown in figure 4.

FIGURE 4.—Sunflower (Mammoth Russian) plants 6 weeks old, grown in sand cultures: *A*, No boron added to nutrient solution; *B*, 2.5 p. p. m. of boron in nutrient solution.

A comparison of the appearance of the plants grown in the sand culture without added boron and of plants grown in similar solutions without the sand (figs. 1, 2, and 3) makes it clear that the plants in this experiment obtained some boron from the sand. The quantity of boron so obtained was evidently small, because all the plants grown in sand without added boron were subnormal in some respects; most of the leaves were distorted in shape, and some of the upper internodes were much shortened. The characteristic condition of the growing point of a boron-deficient plant is shown in figure 5.

The findings of experiment 5, in respect to the growth, boron content, and water used, for the two groups of plants, are shown in table 7. The plants grown without added boron were smaller and contained much less boron than those having an ample supply of that element. They also used less water but had a higher water

taining 2.5 p. p. m. of boron contained 597 p. p. m. of boron in the leaves as compared with 586 p. p. m. for the leaves of plants grown in similar solutions in experiments 1 and 2.

FIGURE 5.—Growing point and upper leaves of a boron-deficient sunflower plant, 6 weeks after germination, in sand culture.

TABLE 7.—*Data on Mammoth Russian sunflower plants of experiment 5, grown for 6 weeks in sand cultures*

[24 plants in each group]

Culture solution and part of plant	Dry material	Boron in dry material		Water used by plants	Computed boron content of water used	Water requirement
	Grams	*Milligrams*	*Parts per million*	*Liters*	*Parts per million*	*Liters ÷ grams*
Solution without added boron:						
Stems	31.6	0.51	16	48.3	0.031	792
Leaves	29.4	.97	33			
Solution with 2.5 p. p. m. of boron:						
Stems	48.2	1.30	27	[illegible]	[illegible]	[illegible]

The plants grown without added boron in the sand cultures of experiment 5 were similar in appearance to those grown in experiments 3 and 4 in solutions containing 0.025 p. p. m. of boron. In respect to the boron content of the dry leaves, the sand-culture plants contained 33 p. p. m. The plants grown in the low-boron solution (0.025 p. p. m.) of experiments 3 and 4 contained respectively 33 and 24 p. p. m. of boron in the leaves. The 24 plants of experiment 5 had access to 30 liters of culture solution estimated to contain 0.3 mg. of boron. This quantity subtracted from the 1.48 mg. found in the plants would leave approximately 1.2 mg. that must have been obtained from the 12 kg. of sand. In other words, it seems probable that the sand contained at least 0.1 p. p. m. of available boron. Attention is drawn to this low ratio of boron to the sand because it seems probable that had the sand contained much more available boron the plants would have absorbed it. Furthermore, it may be pointed out that it would be difficult, if not impossible, with known methods of direct analysis of soil, to measure quantitatively such a small proportion of boron. It is suggested that the use of plants to absorb the "available" boron from large masses of soil may be a useful method of testing soils believed to be deficient in available boron.

SUMMARY

It was desired to determine by experiment (1) whether sunflower plants grown in water-culture solutions without added boron would exhibit symptoms of boron deficiency; (2) what is approximately the concentration of boron in the culture solution above which normal sunflower plants are produced and below which symptoms of boron deficiency appear; and (3) whether the quantity of boron absorbed by sunflower seedlings is directly related to the quantities of boron available in the nutrient solution in the lower range of boron concentration.

The results of the experiments show that, if the culture solutions contain only the boron occurring as impurities in the ordinary c. p. salts used for nutrient solutions (approximately 0.01 p. p. m. of boron), sunflower seedlings do not grow normally and soon die.

It was found that normal sunflower plants may be produced with culture solutions containing approximately 0.05 mg. of boron per liter and that, if the boron concentration of the culture solution is much below that level, growth is restricted and the plants are abnormal in appearance.

With culture solutions containing approximately 0.05 p. p. m. of boron, the quantity of boron absorbed by the plant, as determined by analysis of the plant material, is closely related to the quantity available in the solution.

It is suggested that the quantity of available boron in a sample of soil or in soils in the field may be estimated by growing sunflower seedlings up to 6 weeks old and then analyzing the leaves for boron.

An experiment in which sunflowers were grown in sand cultures indicated that the sand contained approximately 0.1 p. p. m. of boron, a concentration so low as to be difficult to determine quantitatively by direct analysis of the sand.

It was found that throughout the lower range of concentrations of

stems of sunflower plants, with reference to the plant water, was equivalent to approximately 2 p. p. m. The concentration of boron in the leaves was higher and increased progressively with the increase of boron in the culture solution.

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TWO NEW SPORE-FORMING BACTERIA CAUSING MILKY DISEASES OF JAPANESE BEETLE LARVAE[1]

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INTRODUCTION

The existence of diseases among larvae of the Japanese beetle (*Popillia japonica* Newm.) and the part they play in the reduction of the populations of these larvae has been realized for some time. Probably the most important from the standpoint of the natural control of the insect are the so-called milky diseases. Two distinct milky diseases are recognized, referred to hereafter as type A and type B, whose causal agents are two closely related spore-forming bacteria. The author proposes the name *Bacillus popilliae*, n. sp., family Bacillaceae, for the species causing the type A disease and *Bacillus lentimorbus*, n. sp., for the causal agent of the type B disease. A search of the literature relating to insect diseases as well as that on spore-forming bacteria has failed to reveal any forms similar to these two bacilli.

Hawley and White[2] indicated that the diseases of the Japanese beetle could be classified, on the basis of the gross appearance of affected larvae, into three groups, the black group, the white group, and the fungus group. They considered that the majority of the dead larvae found in the field belonged to the black group. They concluded that there was probably only one disease present among larvae of the white group. This disease was characterized by the presence of large numbers of a microorganism in pure or nearly pure culture, which was probably the causal organism.

The writer[3] found that there were several diseases in the white group, of which types A and B milky diseases were the most prevalent and seemed to be responsible for the greater part of the reduction in larval population within the older area of beetle infestation. More recently Hadley[4] has given a brief summary of the status of the disease investigations. The present paper deals with the description of these milky disease organisms.

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[1] Received for publication March 27, 1940. An investigation of the diseases of the immature stages of the Japanese beetle is being carried on jointly by the Bnreau of Entomology and Plant Quarantine of the U. S. Department of Agriculture and the New Jersey Agricultural Experiment Station.

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THE TYPE A MILKY DISEASE ORGANISM

SYMPTOMS OF THE DISEASE

Gross Appearance

To the inexperienced eye there appears to be little difference between healthy larvae and those infected with the type A milky disease. However, even in the early stages the diseased grubs show a turbidity of the blood which obscures the dorsal blood vessel readily seen in healthy grubs. As the disease advances, the grubs acquire a milky-white appearance, which an experienced observer can easily distinguish from the fat accumulation in mature larvae (fig. 1).

A *B*

Figure 1.—Appearance of healthy larvae (*A*) and of larvae with type A milky disease (*B*). × about 2.

The activity of the larvae is not affected until within a few days of death, when they become sluggish. At the same time they turn slightly brownish, except in the lower parts of the body, which become chalky white owing to the settling out of spores as the blood circulation slows down.

Microscopic Appearance

When blood from a diseased larva is observed under the microscope, it is found to be swarming with two types of cells—a highly refractile, spindle-shaped, spore-bearing rod, and a slender, nonmotile rod. These cells are apparently developmental stages of the same organism. Few blood cells are observed, and these few appear little different from those of normal larvae. The milky-white appearance of the blood is due to these highly refractile spores, which may be present in numbers as high as 20 billion in the blood of a single individual (fig. 2). Examination of the fat tissue surrounding the intestine shows a large number of spores. Examination of other tissues by gross dissection does not reveal localization of spores in any number, although some are observed in the layers of cells of the midintestine.

DEMONSTRATION OF THE CAUSAL RELATIONSHIP

The causal relationship between the disease and the organism

a diseased larva was injected into healthy larvae, the typical disease picture appeared. When saline suspensions of blood from a diseased larva were heated to 80° C. for 10 minutes and then injected into healthy larvae, the disease developed. The injected larvae showed the presence of the slender rod-shaped cells a few days after inoculation, and later both spores and rods, with the rods present in all transitional stages between the slender and the swollen spindle forms. These inoculation tests have been made with several thousand larvae

FIGURE 2.—Photomicrograph of the blood of a larva with type A milky disease, showing spore and rod forms of the disease organism. × 1,200.

and seem to establish the etiology of the disease and to show that the rod forms and spore-bearing spindle cells are only developmental stages of the same organism.

DESCRIPTION OF THE CAUSAL ORGANISM

MORPHOLOGY AND STAINING REACTIONS

The vegetative form of the organism is a slender, nonmotile rod occurring singly or in pairs. In the living condition the rods measure 0.9 by 5.2 microns. When fixed by Schaudinn's solution and stained by Hucker's crystal violet, the dimensions are about 0.3 by 3.5 microns.

The mode of division appears to be by plate formation rather than constriction, and is evidenced by the squareness of adjoining ends of the paired cells. After separation the ends are somewhat rounded. The cytoplasm in young cells is homogeneous and stains uniformly with Gram stain; in older cells granules are often found, and after fixing and staining, unstained areas are seen which divide the cell into two unequal sections.

The rods become swollen at sporulation. When the cell begins to swell, the spore becomes visible as a slightly refractile vacuole equal in size to the mature spore. As sporulation proceeds, the vacuole becomes more and more refractile until a definite spore is observed. At this time the cell has a pronounced spindle shape, and the spore is located somewhat terminally. One end of the cell broadens, and the cell becomes more pyriform than spindle-shaped. A granule is now observed in the broadened end, which grows until it is about half the size of the spore. With the development of the granule the spore assumes a more nearly central position. The cytoplasm about the spore becomes increasingly refringent.

After the completion of the refractile body and the increase in density of the cytoplasm surrounding the spore, no further morphological changes occur. In the fresh state the spore and granule are homogeneous in internal structure, and they do not take up either stains or iodine. The spore is surrounded by a halo formed by the encircling protoplasm, but it is very definite in outline. Spores free from the sporangium have never been observed. The size of the unstained sporangium is 1.6 by 5.5 microns, and that of the endospore 0.9 by 1.8 microns. When fixed by Schaudinn's solution and stained with Hucker's crystal violet, the refractile body and spore remain unstained, but the latter is obscured by the deeply stained surrounding protoplasmic layer. When fixed and stained, the spore-bearing cells are approximately 1.3 by 3.6 microns in size. When stained by Dorner spore stain, both the refractile body and the spore retain the stain, whereas the cytoplasm is completely decolorized. The membrane of the vegetative rods and both the membrane and the refractile body of the spore-bearing forms are resistant to the action of alkalies, remaining intact for at least 2 days in 10-percent sodium hydroxide solution.

Germination of the spores has never been observed in either the blood or the digestive fluids of the insect.

Artificial Culture of the Type A Organism

Repeated attempts to isolate the causal organism from the blood of infected larvae were unsuccessful. No evidence of growth, with either the vegetative or the spore stages, was obtained on nutrient agar by shake, slant, or Petri-dish culture methods. The inverted Petri-dish method of Krumwiede and Pratt,[5] which has frequently been used successfully for the isolation and cultivation of anaerobic bacteria, did not yield positive results. On blood-agar slants a slender, motile rod, forming small discrete colonies, was frequently obtained, but attempts to produce the disease by inoculation into healthy larvae were not successful. In peptone-glucose litmus whole milk

[5] Krumwiede, Charles, Jr., and Pratt, Josephine. fusiform bacilli. isolation and cultivation. Jour. Inf. Dis. 12: 199–201, illus. 1913.

heavy inoculations with spore forms yielded a slender, nonmotile rod identical in morphology with the vegetative forms seen in the blood of diseased larvae. No attempts were made to infect healthy larvae with the rods obtained in the milk cultures, since the number of rods produced in the cultures was not much greater than the number of ungerminated spores remaining in the medium.

Recently, when dried-blood films from diseased larvae have been used as the source of spores for inoculation, a large proportion of the attempts to isolate the causal organism have been successful.

Unheated egg-yolk media, used by White[6] for the isolation of *Bacillus larvae* White, proved to be satisfactory for the isolation of the type A milky disease organism. Heavy inoculations of spores from dried-blood films (700,000 spores per 5-ml. slant) were used, and the inoculated slants were incubated at 33° C. Slants of the basal medium, with and without the addition of sterile unheated egg yolk, were incubated under aerobic conditions at atmospheric pressure and at 700-mm. pressure, with the addition of 10 percent by volume of carbon dioxide. Inoculated slants were also incubated anaerobically under a pressure of 100 mm. of carbon dioxide. Under aerobic conditions growth of the type A organism occurred only on the egg-enriched medium, whereas under anaerobic conditions growth occurred equally with and without egg yolk. The beneficial action of the egg yolk must therefore be due in part to reduction of the oxygen content of the medium. The basal medium was fresh beef-infusion agar adjusted to pH 6.8, containing 0.5 percent each of dextrose and peptone. Sterile unheated egg-yolk suspension was added at the rate of 1 ml. per 5 ml. of the basal medium. Beef-infusion agar without peptone and without dextrose was also satisfactory as the basal medium.

The organisms form small discrete colonies on the slants, and as yet only nonmotile slender rods have been obtained in artificial culture. When the rods obtained in pure culture are inoculated into healthy larvae, the typical disease symptoms are produced and the blood of the inoculated larvae becomes loaded with typical spore forms of the type A organism.

Resistance of the Spores

The spores are heat-resistant, withstanding temperatures of 80° C. for 10 minutes, as shown by the production of the disease in larvae by inoculation of heated spore suspensions. The thermal death point of the spores has not been determined. The spores are also resistant to desiccation. Spores in blood films dried for periods as long as 42 months have given consistently high infection when moistened and inoculated into healthy larvae.

Development of the Causal Organism in the Insect's Blood

When healthy larvae are inoculated with spores of the causal organism and held at 30° C., certain developmental changes occur in the bacteria in the blood (fig. 3). For about 12 hours after inoculation no change is detected in either the morphology or the number of spores. Then there is a gradual decrease in the number of spores

[6] White, G. F. American foulbrood. U. S. Dept. Agr. Bul. 809, 46 pp., illus. 1920.

until, after 30 hours, about half the original number remain. At this time vegetative forms are also seen in small numbers; they usually occur in pairs (*C*, *D*). After 48 hours about one-third of the original spores remain, and rods are present in extremely large numbers, still largely in pairs. On the third day after inoculation the rods begin to swell and many cells show the presence of an oval central vacuole (*E*, *F*). During the next 24 hours there is an increase in refringency of the vacuole with the formation of the mature endospore (*G*, *H*). At this time most of the rods are observed in the early stages of sporulation; there is a slight bulging of the rod with the appearance of the vacuole, and a few cells with well-developed endospores, swollen

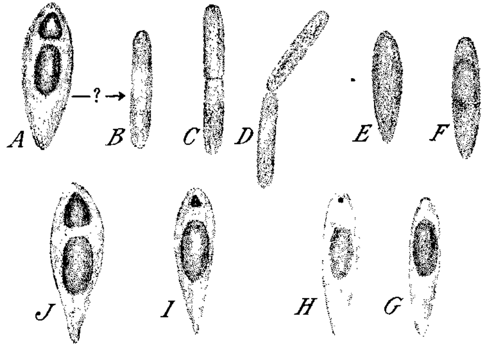

FIGURE 3.—Drawing illustrating the mode of reproduction and sporulation of the type A milky disease organism in the blood of the Japanese beetle larva: *A*, Parent spore; *B–D*, reproducing rods; *E–I*, sporulating rods in various stages of spore formation; *J*, mature spore-bearing rod. The question mark between *A* and *B* denotes that the exact mode of germination of the spores has not yet been determined. × about 6,000.

considerably and somewhat pyriform, showing a small granule in the broader pole (*I*). Periodic examination during the next 24 hours shows an increasing number of cells with initial granule formation and a few cells with the granule enlarged to the well-developed refractile body (*J*). The cells have the general appearance of those originally injected. By the sixth day the spores are sufficiently numerous so that the turbidity of the blood may be observed as a change in the external appearance of the larva. At this time many of the rods are still present, in the state both of division and of sporulation. The number of spores reaches a maximum about 7 to 10 days later, and at this time the larva is distinctly milky in appearance. The blood is found to be swarming with mature spore-bearing cells, and the rods, which are still present in considerable number, appear granular and many have lost their refringency. These so-called shadow cells are probably for the most part incapable of sporulation. The total num-

ber of spores in the blood at this time is from 2 to 20 billion, averaging about 5 billion, per larva. Subsequent examinations until the larva dies do not show any marked changes in the appearance of the spores, except that the surrounding protoplasmic layers are somewhat thicker and more refringent. Most of the rods appear to be either shadow cells, excessively granular cells, or sporulated cells that are not swollen appreciably. The rods stain only feebly. Examination of droppings from diseased larvae or the contents of the mid- or hind-intestine has not revealed the presence of spores or typical vegetative rods.

EFFECT OF TEMPERATURE ON TIME OF DEVELOPMENT OF DISEASE

To determine the effect of temperature on the time of development of macroscopic symptoms of the disease, larvae were injected with 2 million spores and held at various temperatures. The times of development were as follows: 4 days at 34° C., 6 days at 30°, 9 days at 25°, 11 days at 22°, and 14 days at 17°. Larvae held at 13° were not diseased after 63 days, and those held at 9° were still healthy after 28 days. When the larvae held at 9° for 28 days were placed at 30°, they developed the disease after 5 days.

The foregoing data show a linear relationship between the time of development of the disease and the temperature between 17° and 34° C. This corresponds approximately to the mathematical expression $T=24-0.6\,\theta$ (°C.), where T is the time of development of the disease and θ is the temperature of incubation.

Other tests were run with inoculated larvae held at 36°, 37°, and 40° C., for comparison with larvae similarly inoculated and held at 30° as checks. Although the checks showed consistent disease development, in no case did larvae held at the higher temperatures develop the disease. Temperatures as high as 36° to 40° are close to the maximum tolerated by larvae, and in no case did larvae survive after 1 week. In view of the rapid development of the disease at 34°, lack of development after 7 days at higher temperatures seems to indicate that 36° must be approximately the maximum temperature for development of the causal organism.

Larvae held at temperatures of 15° to 16° C. had not developed the disease after 29 days. The blood of larvae held at 15° for 29 days was found to contain a few spores but no vegetative forms. From these observations it is concluded that the most probable temperature range for development of this disease is from 16°–17° to 36°.

THE TYPE B MILKY DISEASE ORGANISM

SYMPTOMS OF THE DISEASE

Gross Appearance

Larvae infected with type B milky disease found in the late summer and fall cannot be distinguished macroscopically from those infected with the type A disease. In overwintering diseased larvae, however, the general appearance is quite different. Instead of having a milky-white coloration, these larvae are a muddy brown. Overwintering diseased larvae collected in March were milky white with little or no brownish discoloration but when held at room temperature they

darkened rapidly until at the end of 2 or 3 weeks they had assumed the chocolate color generally found in type B-diseased larvae during April and May. Microscopic examination has shown this darkening of diseased larvae to be due to extensive formation of blood clots which are brown to jet black. Chocolate-brown larvae are still alive and active (fig. 4). The accumulation of these clots in appendages blocks the blood circulation, producing a gangrenous condition which

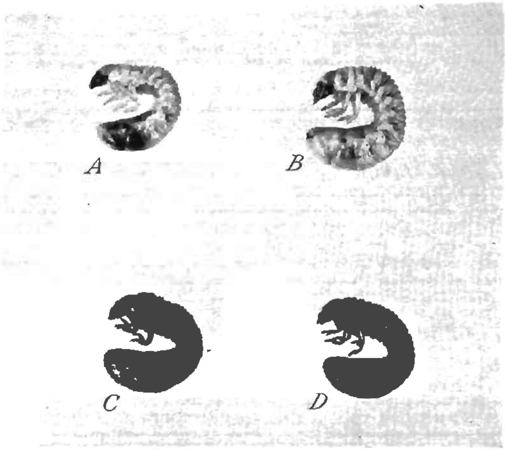

FIGURE 4.—Photograph of living larvae showing darkening of the overwintering type B milky-diseased larvae due to clot formation: *A*, Normal larva; B, overwintering diseased larva with clotting still quite limited; *C*, *D*, overwintering diseased larvae showing extensive clot formation (note the darkening of the legs caused by massing of the spores). × about 2.

causes the affected parts to blacken. Death of such larvae is probably the result of gangrene.

When healthy larvae injected with blood of the brown diseased larvae develop the disease, they show the milky-white condition rather than the brown coloration of the larvae used as inocula.

Microscopic Appearance

The blood of diseased larvae shows the presence of large numbers of spindle-shaped spore-bearing rods and nonmotile vegetative rods (fig. 5). In blood of overwintering larvae the rods are nonrefractile shadow cells, and many of the spore-bearing rods show thickened and darkened membranes, which appear to be encysted by precipitation of blood about the spores. In addition, a large number of irregu-

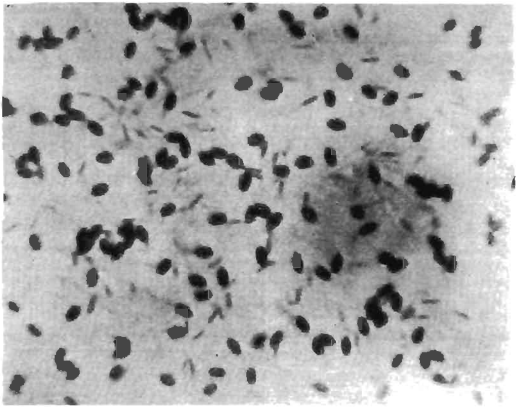

FIGURE 5.—Photomicrograph of blood of a larva with type B milky disease. Note the deeply stained spores and slender rod forms. × about 1,200.

DEMONSTRATION OF THE CAUSAL RELATIONSHIP

The disease is readily produced in healthy larvae by the injection of either heated or unheated suspensions of the blood of diseased larvae. Injection of either spores or vegetative rods produces the same sequence of events—namely, the appearance, first, of a large number of vegetative rods, followed by the typical spindle-shaped spore-bearing forms and the milky-white coloration of the larvae.

DESCRIPTION OF THE CAUSAL ORGANISM

MORPHOLOGY AND STAINING REACTIONS

The morphology and staining reactions of the vegetative form of the type B milky-disease organism are similar to those of the type A organism. The spore-bearing forms, however, although having considerable resemblance, are easily differentiated. The refractile body, so prominent in the type A sporangium, is absent in this form, and the sporangium is more decidedly spindle-shaped. The morphological differences apparent in the living spores are just as pronounced in fixed and stained spores. In the type B organism the spore-bearing rods take up crystal violet strongly and evenly, and the stained sporangium has a distinct lemon shape (fig. 6).

The dimensions of the vegetative rod are about 1.0 by 5.0 microns in the living state, and 0.5 by 4.0 microns when fixed by Schaudinn's solution and stained with crystal violet. The dimensions of the endospore are 0.9 by 1.8 microns, and of the sporangium 1.4 by 3.9 microns. When the sporangium is fixed and stained, its apparent dimensions are 1.9 by 2.8 microns.

Both rods and spore-bearing forms are stained by Hucker's modification of the Gram stain. The spores retain a deep red, and the sporangia are decolorized when stained by the Dorner method.

Attempts at artificial culture and isolation of the causal organism have thus far been unsuccessful.

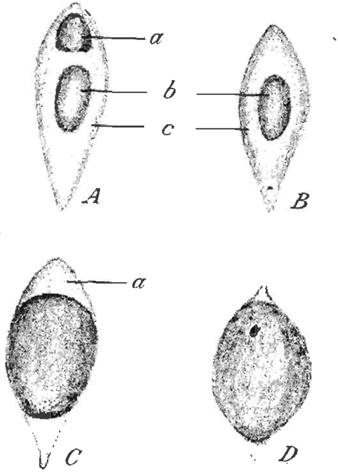

FIGURE 6.—Drawings illustrating the major morphological differences between the type A and type B spores: *A*, Type A, and *B*, type B spore unstained: *a*, Refractile body; *b*, endospore; *c*, sporangium. Note the absence of a refractile body and greater symmetry in *B*. *C*, Type A spore stained with crystal violet: *a*, Position of unstained refractile body. Note the lack of uniformity in staining. *D*, Type B spore stained with crystal violet. Note the uniform staining and lemon shape. × about 10,000.

RESISTANCE OF THE SPORES

The spores are resistant to heat, withstanding at least 85° C. for 10 minutes when heated in physiological saline suspensions. They are also resistant to desiccation, producing the disease upon injection into the healthy larvae after drying in thin blood films on glass slides for as long as 42 months.

DEVELOPMENT OF THE ORGANISM WITHIN THE HOST

Larvae were inoculated with 2 million spores by injection with spore suspensions of the causal organism, and held at 30° C. As with the type A disease, the organism is largely a blood parasite, although other tissues may be attacked. Periodic examination of

the blood showed the following changes in the organism: For 2 days after inoculation a gradual reduction in the number of spores was observed. On the third day vegetative rods appeared in considerable numbers, most of which were present in pairs. Adjoining ends of paired cells were truncate, indicating that the division probably occurred by plate formation rather than by constriction. The number of rods increased with time, and swelling of the rods began on the fifth day. At that time the presence of vacuoles was also noted in a few cells. On the sixth, seventh, and eighth days there was a growing preponderance of swollen sporulating rods, until at the ninth day they were present in sufficient numbers to give the first external symptoms of the disease.

At 30° C. development of the causal organism seems to be retarded, and the number of spores per larva seldom exceeds 1 to 2 billion even after 2 weeks at this temperature. At temperatures lower than 30° an increase in the number of spores continues after visible symptoms are observed, and after 2 to 3 weeks the typical milky-white condition of field-collected diseased larvae is reached. At this time between 5 and 10 billion spores per larva are present. Mature third instars, inoculated by injection, frequently had pupated before the disease caused their death.

EFFECT OF TEMPERATURE ON DEVELOPMENT OF THE DISEASE

When larvae were inoculated by injection and held at different temperatures, both the time and the extent of development of the disease were different. As mentioned above, although the time of development of the first external symptoms is the same (9 days) at 30° and 25° C., the extent of development is less at the higher temperature. At 22° external symptoms first appeared after 10 days, and at 15.5° after 19 days; at 12° there was no development after 63 days, and spores were in evidence but no rods.

The absolute maximum and minimum temperatures for development have not been determined for the type B organism, but the observations between 12° and 30° C. indicate that 30° is close to the maximum temperature, since the disease develops less than at lower temperatures, and that the minimum temperature is between 12° and 16°, probably closer to the latter. It seems, therefore, that the optimum and maximum temperatures for development of the type B disease are lower than those for the type A disease, and the minimum temperature for development is very nearly the same for both diseases. Type B disease thus has a considerably smaller temperature range than type A disease.

SUMMARY

Two new spore-forming bacteria are described. These organisms are the causal agents of types A and B milky disease of the larvae of the Japanese beetle (*Popillia japonica* Newm.).

The type A milky disease organism is a nonmotile Gram-positive rod measuring about 0.9 by 5.2 microns. The rods become swollen at sporulation, assuming first a spindle and then a pyriform shape. The spores are cylindrical and measure about 0.9 by 1.8 microns and are located centrally in the cell. In the broader pole of the cell

is found a refractile body, which is about half the size of the spore and possesses staining reactions similar to those of the spore. The temperature range of development seems to be from 16° to 36° C. The spores are found mainly in the blood of the larvae, reaching numbers as high as 20 billion in a single insect.

The type B organism is similar in appearance to the type A organism in the vegetative stages, but is readily distinguished morphologically after sporulation. The refractile body so prominent in the type A spore is lacking in the type B organism, and the spore-bearing rods are more nearly spindle-shaped. The temperature range of development seems to be somewhat narrower than for the type A organism, although the minimum temperature for development is the same (16° C.).

Both organisms produce a similar disease condition in the larvae of the Japanese beetle, so that upon gross examination the two conditions are usually indistinguishable.

Only the type A organism has thus far been cultured on artificial media.

The author proposes the name *Bacillus popilliae*, n. sp., family Bacillaceae, for the species causing the type A milky disease and *Bacillus lentimorbus*, n. sp., for the species causing the type B disease.

COLOR MARKINGS IN RHODE ISLAND RED CHICKS AS RELATED TO SEX AND ADULT COLOR[1]

By F. A. Hays

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INTRODUCTION

Rhode Island Red chicks have long been known to vary widely in the color of their down. Warren[2] made a study of the inheritance of down color in Rhode Island Red chicks and its relation to adult plumage color and pointed out important variations in down color. Although he was not concerned with possible relations between down color and sex, he noted that striped chicks were preponderantly females.

Byerly and Quinn[3] made a study of down color in Rhode Island Red chicks in relation to sex. They studied 1,102 chicks and well-developed embryos from production-bred stock and 663 chicks from a standard-bred flock. They classified chicks for black pigment spots on the head or stripes on the back, but they made no reference to brown pigmented areas on head or back. Their data showed that 47.5 percent of the chicks examined were spotted or striped and that 84.9 percent of the spotted or striped chicks were females and 77.8 percent of the nonspotted were males. Of the striped chicks 93.6 percent were females. In standard-bred chicks 42.1 percent were either spotted or striped. Byerly and Quinn concluded that spotting and striping are widely distributed in Rhode Island Red chicks.

Quinn and Byerly[4] made a further study of spotting and striping in Rhode Island Red and New Hampshire chicks exhibited at the Northeastern Poultry Producers Exposition in 1936. These chicks represented groups selected for uniformity in down color. Of a total of 650 chicks from 26 different entries, 24.9 percent of the Rhode Island Reds and 13.3 percent of the New Hampshires showed some melanic pigment. The sex was determined on five lots of chicks from each breed and in this composite sample only 19.4 percent showed any melanic pigment. A sex ratio of 142 males to 100 females was observed. The percentage of females in the spotted chicks was 78.8. New Hampshire chicks were freer from melanic pigment than Rhode Island Reds both in selected and unselected samples.

EXPERIMENTAL METHODS

Color markings were recorded for 8,713 Rhode Island Red chicks as they were taken from the incubator in 1937, 1938, and 1939. Most of these chicks were bred for high fecundity, but a small number bred for exhibition quality were included each year. Distinction was

[1] Received for publication March 18, 1940. Contribution No. 368 of the Massachusetts Agricultural Experiment Station.

[2] Warren, D. C. the inheritance of rhode island red chick down-color variations and their relation to color variations in adult plumage. Jour. Agr. Res. 39: 781–794, illus. 1929.

[3] Byerly, T. C., and Quinn, J. P. sexual dimorphism in single comb rhode island red down color. Jour. Hered. 27: 319–322, illus. 1936.

[4] Quinn, J. P., and Byerly, T. C. spotting and striping in exhibition classes of rhode island red and new hampshire baby chicks. Poultry Sci. 16: 422–425. 1937.

made between brown and black pigment areas, and the location of the pigmented area was indicated. No attempt was made to breed for or against spotting and striping so that the data actually represent an unselected population of pedigreed chicks. Adult color was taken at 6 months of age on part of the birds and the color classes used represent the general surface color.

DOWN COLOR OF CHICKS WHEN HATCHED

The down color of the chicks according to sex is recorded in table 1. Table 1 shows that in the male chicks 89.84 percent had no color markings; about 10 percent had either black or brown spots or stripes on the head; about 0.2 percent had black on the neck, 0.09 percent had brown on the neck; and 0.38 percent had black on the back as compared with 0.16 percent with brown on the back.

Among the female chicks 55.86 percent had solid down color and 44.14 percent had some type of spotting or striping as compared with about 10 percent of marked chicks among the males. In the total population of 8,713 chicks the probability of any one chick being a marked female was only 0.2159 and the probability of a marked chick being female was 0.806. The data show further that female chicks are much more likely to have pigment stripes on neck and back, as Warren [5] has pointed out.

TABLE 1.—*Color markings of 8,713 male and female chicks when hatched*

Color marking	Males		Females	
	Number	*Percent*	*Number*	*Percent*
None	3,997	89.84	2,382	55.86
Black head	198	4.45	1,175	27.56
Brown head	250	5.62	703	16.49
Black neck	9	.20	55	1.29
Brown neck	4	.09	19	.45
Black back	17	.38	106	2.49
Brown back	7	.16	56	1.31
Chicks observed	[1] 4,449	----	[1] 4,264	-

[1] Several chicks appear more than once because pigmentation occurred in more than 1 area.

DISTRIBUTION OF PIGMENT IN BLACK AND BROWN PIGMENTED CHICKS

A comparison of black and brown pigmented chicks of the same sex is shown in table 2 for the 2,325 pigmented chicks.

TABLE 2.—*The relation of sex to distribution of color markings in black and brown pigmented chicks* [1]

Body part	Black pigmentation				Brown pigmentation			
	Male		Female		Male		Female	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Head only	176	88.44	1,024	87.22	242	96.03	633	90.43
Neck only	0	0	0	0	1	.40	0	0
Back only	2	1.01	3	.26	1	.40	1	.14
Head and neck	6	3.02	47	4.00	3	1.19	17	2.43
Head and back	13	6.53	93	7.92	5	1.98	47	6.71
Head, neck, and back	2	1.01	7	.60	0	0	2	.29
Total	199	-----	1,174	---	252	-------	700	-----

[1] 9 chicks recorded as having black in one part of the body and brown in another are omitted.

[5] See footnote 2.

When black pigment appeared in the individual there was no sex difference in its distribution. About 88 percent of males and 87 percent of females exhibited their black pigment on the head. Head and back showing black pigment in the shape of a head spot and a stripe on the back was next in importance and equally prevalent in the males and females that carried down pigmentation. Black pigment occurring as a stripe from the head along the neck was of considerable importance and probably about as frequent in males as in females in pigmented individuals. Black pigment did not appear on the neck alone; it did sometimes appear on the back alone. About 1 percent of black-pigmented chicks showed the black on head, neck, and back simultaneously.

Brown head spots were more prevalent in males than were black head spots. Brown on head and back was three times as prevalent in females as in males. Brown stripe on head and neck was more prevalent in the females. Brown pigment seldom appeared on the neck or back alone and very seldom on head, neck, and back simultaneously.

The data in table 2 indicate that black pigment spots and stripes were more common in the population studied than were brown pigmented areas. There is further evidence that some sex differences may occur in the distribution of brown pigment.

DISTRIBUTION OF COLOR MARKINGS OF CHICKS IN THE TOTAL POPULATION

A study was next made of the distribution of black and brown pigment without regard to sex in those chicks that carried pigmented spots or stripes at hatching. Table 3 records the combined data on 2,325 chicks.

TABLE 3.—*Distribution of color markings of 2,325 newly hatched black and brown pigmented chicks of both sexes* [1]

Body part	Black pigmentation			Brown pigmentation		
	Chicks	Proportion of all chicks	Proportion of males	Chicks	Proportion of all chicks	Proportion of males
	Number	*Percent*	*Percent*	*Number*	*Percent*	*Percent*
Head only	1,200	13.77	14.67	875	10.04	27.66
Neck only	0	0	---	1	.01	100.00
Back only	5	.06	40.00	2	.02	50.00
Head and neck	53	.61	11.32	20	.23	15.00
Head and back	106	1.22	12.26	52	.60	9.62
Head, neck, and back	9	.10	22.22	2	.02	0
Total	1,373	15.76	14.49	952	10.93	26.47

[1] 9 chicks recorded as having black in one part of the body and brown in another are omitted.

Of the chicks that showed black on the head only 14.67 percent were males, indicating that about 85 percent of the chicks with black pigment on the head were females, an observation which agrees closely with the findings of Byerly and Quinn.[6] When the head spot was brown 27.66 percent of the chicks were males. The appearance of black spots or stripes on head, neck, or back, or various combinations of these areas, showed about the same relation to sex. When the

[6] See footnote 3.

pigment was brown it generally appeared on the head and was somewhat less closely associated with sex. Chicks marked with black represented 15.76 percent of all chicks, and those marked with brown represented 10.93 percent of all chicks. There were, therefore, 26.69 percent of all chicks that showed some color markings. From the total of 8,713 chicks there were only 26.69 percent whose down pigmentation could be used as a guide in separating sex. Considering the marked chicks only, 14.49 percent of those marked with black were males and 26.47 percent of those marked with brown were males. When all chicks marked with black were combined with all marked with brown, 19.40 percent were found to be males and 80.60 percent females. As a practical means of separating sexes, down spots and stripes are of little value.

RELATION OF DOWN COLOR MARKINGS TO ADULT COLOR

An attempt was made to discover any relations that might exist between black and brown down color markings and general adult plumage color. For this purpose it was considered advisable to classify the birds at 6 months of age for general color of surface plumage rather than for color in different body regions. Adult plumage color was grouped into 5 general classes as shown in table 4 and male and females were considered separately and combined. Adult colors were recorded during the last 2 years of the study on 1,295 males and on 2,493 females. The data are summarized in table 4.

TABLE 4.—*Color markings of chicks at hatching as related to adult plumage color and to sex, 1938 and 1939*

Sex and adult color	Adults which, as chicks, were—						Total	
	Nonmarked		Black-marked		Brown-marked			
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Males:								
Very light red	21	1.73	0	0	0	0	21	1.62
Light red	684	56.39	20	37.04	13	46.43	717	55.37
Medium red	341	28.11	17	31.48	11	39.29	369	28.49
Dark or standard red	125	10.31	16	29.63	3	10.71	144	11.12
Black mottled	42	3.46	1	1.85	1	3.57	44	3.40
Total	1,213		54		28		1,295	
Females:								
Very light red	76	5.13	34	4.70	25	8.65	135	5.42
Light red	784	52.94	312	43.15	153	52.94	1,249	50.10
Medium red	451	30.45	233	32.23	92	31.83	776	31.13
Dark or standard red	122	8.24	128	17.70	9	3.11	259	10.39
Black mottled	48	3.24	16	2.21	10	3.46	74	2.97
Total	1,481		723		289		2,493	
Both sexes:								
Very light red	97	3.60	34	4.38	25	7.89	156	4.12
Light red	1,468	54.49	332	42.73	166	52.37	1,066	51.90
Medium red	792	29.40	250	32.18	103	32.49	1,145	30.23
Dark or standard red	247	9.17	144	18.53	12	3.79	403	10.64
Black mottled	90	3.34	17	2.19	11	3.47	118	3.12
Total	2,694		777		317		3,788	

Of the 1,213 males that had no pigment spots when hatched, 6.39 percent were classed as light red in adult plumage and 28.11

percent as medium red. Slightly over 10 percent approached the dark or standard red in adult color, and 1.73 percent were very light red. Black mottling appeared in 3.46 percent of the nonmarked males when they developed their adult plumage.

The small group of male chicks that had some black markings exhibited a somewhat darker adult plumage than was observed in nonmarked chicks. About 61 percent of these black-marked chicks showed medium or dark adult plumage color. Only 37 percent of these males marked with black were recorded as light red in adult plumage and none as very light red. Black mottling was less common also in the black-marked males.

A very small number of male chicks had brown spots or stripes. There was some evidence that brown spotting or striping was associated with light adult plumage color. The total male population showed light red and medium red as the prevailing adult color.

Female chicks that were not marked showed a higher percentage of very light red individuals than the males and a somewhat smaller percentage of dark red. The incidence of black mottling was almost the same in both sexes. The data suggest in general that the prevailing shade of adult color was slightly lighter in females than in males on a relative basis.

Black-marked female chicks developed a little darker adult color than did the nonmarked females. The percentage of dark-red birds among the black-marked females was twice as large as that among the nonmarked females. The percentage of light-red individuals was smaller in the black-marked group and black mottling was somewhat less frequent.

Brown-marked female chicks tended to run a little lighter in adult plumage than nonmarked or black-marked chicks. There was a very small percentage with dark plumage color. Black mottling was equally frequent in brown-marked and nonmarked chicks. Adult males were slightly darker than females. In both sexes the appearance of black pigment in down appears to be associated with darker shades of general plumage color in adults. Differences observed in the adult color of black-marked chicks as compared with nonmarked or brown-marked chicks are not outstanding but do appear to exist.

In the total population light-red adult color prevails in the three kinds of chicks. There again appears to be an advantage from the standpoint of darker adult color and freedom from mottling in selecting chicks with black pigmented areas. In a flock bred for high fecundity where the general plumage color tends to run light, something may be gained in plumage color by selecting chicks that show black pigment spots or stripes.

SUMMARY AND CONCLUSION

A study of 8,713 chicks largely bred for egg production was made to determine the relation of down pigmentation to sex and to adult color.

Only about 10 percent of male chicks showed any color markings in the down as compared with about 44 percent of female chicks that showed spotting or striping. In the total population only 26.76 percent of the chicks had spots or stripes in the down.

In the male chicks 4.47 percent had black pigmented areas and 5.66 percent had brown pigmented areas; in the females 27.53 percent had black pigmented areas and 16.62 percent had brown.

The prevailing type of pigment area was a spot on the head. In the population with pigmented down, black was confined to the head as often in males as in females. Brown pigment spots were slightly more common in male chicks in the population showing pigment spots. When chicks were striped the color of pigment was more likely to be black.

Considering only the marked population, about 15 percent of the chicks with black pigment were males and about 26 percent with brown pigment were males.

Adult color in males averaged slightly lighter than in females.

Chicks of both sexes that carried some black pigmented areas developed a slightly darker adult plumage color and fewer mottled individuals than chicks with solid down color or with brown pigmented areas.

Color markings in down has no commercial value as an indicator of sex in the stock studied.

THE SIGNIFICANCE OF THE "CEASED" REACTOR TO BANG'S DISEASE [1]

By B. A. Beach, *professor of veterinary science, Wisconsin Agricultural Experiment Station;* M. R. Irwin, *cooperative agent, Bureau of Animal Industry, and professor of genetics, Wisconsin Agricultural Experiment Station;* and L. C. Ferguson, *cooperative agent, Bureau of Animal Industry, and instructor in veterinary science and genetics, Wisconsin Agricultural Experiment Station.*

INTRODUCTION

Are "ceased" reactors (i. e., those animals that have lost their agglutinin titer to *Brucella abortus* following infection) a menace to the herds in which they are kept? This question presents an important practical problem to the owners of the many herds infected with the organism producing Bang's disease. In the Federal-State Bang's disease program for eliminating reactors (i. e., those whose sera agglutinate the causative organism at an appreciable titer) from the participating herds, the problem is also of importance since in many of these herds there undoubtedly are some animals that were once reactors, but cannot now be surely recognized as such. An investigation was made to find an answer to the question about ceased reactors, and the results are reported in the present paper.

MATERIALS AND METHODS

The ceased reactors were observed in three different herds of cows, Nos. 1, 2, and 3, each of which had been artificially exposed to *Brucella abortus.*

Herd 1 was assembled in 1926, and originally consisted of 44 grade and purebred Holstein calves. The pregnant animals, 37 individuals out of the original 44, were exposed to *Brucella abortus* in 1928, with results as previously described by Hart and his coworkers.[2]

After observations had been made on these individuals over a period of 2 years following their exposure, 14 were chosen as ceased reactors. (All the other animals of this herd were slaughtered and examined for the presence of *Brucella abortus.*) However, the serum of cow No. 40, cited in table 1, had reacted only slightly with *Brucella abortus* at a dilution of 1:200, that of Nos. 4, 5, and 31, had reacted slightly at a dilution of 1:100, while that of No. 18 had never shown a definite agglutination with this organism.

The object of the writers in including these five animals in the experiment and in presenting the data in the table, along with those of herd 2 whose sera gave only a low titer, or none, in agglutination of the pathogen, was because of the possibility that such animals might be carriers of the organism. (For example, after exposure of the cows of herd 3, the serum of one animal, 20C, never showed definite agglutina-

[1] Received for publication December 4, 1939. From Paper No. 251, the Department of Veterinary Science and the Department of Genetics, Wisconsin Agricultural Experiment Station; investigation carried out in cooperation with the Bureau of Animal Industry, U. S. Department of Agriculture.

[2] Hart, E. B., Hadley, F. B., and Humphrey, G. C. THE RELATION OF NUTRITION TO CONTAGIOUS CATTLE ABORTION. Wis. Agr. Expt. Sta. Res. Bul. 112, 45 pp., illus. 1932.

tion of the pathogen. Furthermore, she produced a normal calf at full term, yet guinea-pigs inoculated with her uterine fluid gave evidence of the presence of the organism.) It is a point of interest that the organism was recovered from 9 of these 14 cows, following examination of their aborted fetuses, fetal membranes, and colostrum by the usual method of guinea-pig inoculation.

TABLE 1.—*The extremes of the titer of agglutinins and the dates on which these first appeared, for 28 cows in 3 herds whose serum wholly or nearly ceased to react with Brucella abortus, after infection was artificially induced by different methods*

Cow No.	Method of exposure	Serum reaction: Highest titer and time first observed — Titer [2]	Serum reaction: Highest titer and time first observed — After number of months shown	Serum reaction: Time when titer disappeared, or nearly so — After number of months shown
	HERD 1, EXPOSURE BY FEEDING [1] AND CONTACT WITH NATURALLY INFECTED COWS, 1928			
4	Several feedings, September to November	[3]S-1:100	2	3
5		S-1:100	4	6
8		1:200	3	6
12		1:200	1	24
17		1:200	4	6
18		(4)		
28		1:100	1	24
31		S-1:100	3	6
33		1:200	3	24
35		1:200	3	24
36	Contact	1:100	5	7
38	Several feedings, September to November	1:200	3	6
40		S-1:200	4	5
44		1:100	1	6
	HERD 2, EXPOSURE BY INSTILLING A VIRULENT CULTURE INTO THE EYE, AND CONTACT WITH A NATURALLY INFECTED COW, 1933 [5]			
[6]23A	Eye installation and natural exposure	(4)		
32A		1:100	3	16
58A		1:50	2	5
63A		1:25	1	5
9B		1:25	3	5
10B		1:50	2	4
15B		1:100	2	5
	HERD 3, EXPOSURE BY FEEDING, EXCEPT AS NOTED			
3C	Feeding of approximately 142.109 organisms	1:400	4	19
12C		1:200	3	21
20C		(4)		
24C		1:200	1	9
33D		1:400	4	24
4H		1:100	2	3
38D	Instillation into eye	1:200	2	8

1 Stomach contents from aborted fetuses and cultures were used.
2 Unless otherwise stated, the titers represent complete agglutination of the organism at the stated dilutions of the serum.
3 S indicates a slight reaction at the dilutions shown.
4 Either always negative or only weak agglutination.
5 The cow that developed mastitis after being put into herd 3, is not included.
6 This cow had previously passed through an out break of Bang's disease.

Herd 2 was assembled in August 1931, and consisted of the 14 ceased reactors from herd 1 and 22 cows, mostly virgin heifers (normals).

had any previous contact with *Brucella abortus*, except 1 cow (No. 23, A table 1) that had passed through an outbreak of *Br. abortus* some years previously. The serum of 1 of these 22 cows showed the presence of agglutinins for the organism about 2 months after the herd was brought together, and this cow was immediately removed.

All the other animals, both ceased reactors and normals, calved once, a few twice, in the period between the assembly of the herd and the artificial exposure. Five animals, one ceased reactor and four of the normal group, were for various reasons removed from the herd before the artificial exposure to *Brucella abortus*. Three normal, pregnant cows were added to the herd approximately 3 months before the exposure. All animals which had been diagnosed as pregnant were artificially exposed by way of the eye in September 1933. Those which were exposed for the first time and whose sera gradually lost their agglutinins for *Br. abortus*, eight in all, were considered to be ceased reactors. The remaining animals of the herd were slaughtered, and examination was made of each for the presence of the infecting organism.

With the 8 ceased reactors from herd 2 (table 1) as a nucleus, a repetition of the experiment was begun in August 1934. Twenty-eight virgin heifers were purchased from herds free of Bang's disease and placed with the 8 ceased reactors, these together constituting herd 3. One of the 8 individuals developed acute mastitis early in the experiment and was removed; therefore, only 7 individuals are listed in the table. During the course of the experiment, 5 normal cows were added to the herd in April 1936, and 11 were removed at various times and for different reasons. Each of these 16 individuals, however, calved at least once while in the herd, thus having had ample opportunity for contact with the 7 ceased reactors. Each of the other cows calved at least once, the majority twice, and a few three times, before the artificial exposure. Two abortions were observed among the normal animals. These, however, presumably were not caused by *Brucella abortus*, since agglutinins for the organism were not found in the serum of either of the two cows, and negative results were obtained by guinea pig inoculation and culture of the placenta and uterine fluid from each.

The individuals that were diagnosed as pregnant were artificially exposed to *Brucella abortus* in May 1937. The majority of those undergoing their first contact with the pathogen were fed a massive dose of the organism, while the ceased reactors were exposed by instilling a given amount of the culture into the eye.

In both of these herds (Nos. 2 and 3), the ceased reactors and normal cows were placed together in the same barn, barnyard, and pasture. While in the stable, the normals were so placed as to give as much contact as possible with the ceased reactors. As stated, a few animals were removed from these herds during the experiment, usually because of failure to become pregnant. Any replacements that were made are mentioned only if they had contact while pregnant with the ceased reactors.

Tests to determine the agglutinin titer of the serum of all animals in herds 2 and 3 were usually made at monthly, often at weekly, intervals. Both plate and tube methods of determining the titer of the serum were used, with reasonably close agreement in the titer as

found by the two methods. Independent readings of different samples of sera were often made by different workers.

Throughout the course of these experiments (i. e., beginning with the assembly of herd 2 in 1931), in order to determine whether the ceased reactors were carriers of the organism, the colostrum and either the placenta or the uterine fluid, or both, were tested at each parturition for the presence of *Brucella abortus* by the usual method of guinea-pig inoculation.

RESULTS

HERD 2

A summary of observations on the ceased reactors in herd 2 has already been reported,[3] and only a brief mention of certain of the findings will be made here. The titer for *Brucella abortus* of the sera of the 14 ceased reactors of this herd was determined monthly, or oftener, during the period from the beginning of the experiment in 1931 to the artificial exposure of all the pregnant animals of the herd in 1933. Eleven of the ceased reactors were negative, or at most suspicious, in their reaction to the organism during this period. The sera of 3 of these individuals at times gave a low reaction. Further, injection into guinea pigs of the colostrum from each quarter of the udder and uterine fluid from each of these 14 cows failed to reveal *Br. abortus*, except in one instance, when from 1 cow, at 1 freshening only, *Br. abortus* was recovered from the colostrum from 1 quarter of the udder.

During these tests it was noted that the serum from the colostrum from each of these cows showed the presence of antibodies (agglutinins) for the organism at a dilution of 1:400, or higher. This titer, however, disappeared after a few milkings. This finding suggests that there had been a concentration of antibodies in the udders during the nonlactating period.

Shortly after the assembly of herd 2, the serum of one of the normal individuals showed a low titer (1:100) for the organism. This animal was immediately removed from the herd and kept in isolation until after calving. The titer of her serum receded slightly, and the organism was not recovered at calving (normal), following the usual guinea pig injections, or from the body organs when the cow was slaughtered shortly after calving. Except for this one individual, there was no indication of an increase in titer of the serum of the individuals of this group which presumably were noninfected when introduced into the herd.

The exposure of the cows in herd 2 was accomplished by instilling virulent cultures into the eye, and also by introducing an infected cow into the herd. There were 20 cows pregnant at that time that had not previously been exposed to the organism. Following the exposure, the serum of 17 of these showed an agglutinin titer for *Brucella abortus* of 1:50 or higher. The serum of 5 of these 17 cows gradually lost agglutinins for the pathogen. These 5, together with 3 others (9B, 23A, and 63A in table 1) that never showed more than a suspicious reaction, if any, made up the 8 ceased reactors which later were placed

[3] BEACH, B. A., and HUMPHREY, G. C. THE PRESENCE OF BR. ABORTUS IN THE UTERINE FLUID AND IN THE MILK AND OF AGGLUTININS IN THE BLOOD SERA OF SO-CALLED CEASED REACTOR COWS. Vet. Med. 30: 8–10. 1935.

in herd 3. It will be noted, therefore, that 5 out of 17 reactors, or approximately 29 percent, ceased to react. These 5 cows had normal calves after exposure. One of the cows which had given a suspicious agglutination reaction aborted; the other 2 calved normally.

HERD 3

Herd 3, as previously explained, was started on the experiment in August 1934. From the time the herd was assembled to the time that the pregnant individuals were artificially exposed in the late spring of 1937, all the animals remained negative or nearly so, in their agglutination reaction. Only occasionally the serum of one or more of the seven ceased reactors showed a low agglutinin titer.

In this herd, as in herd 2, the colostrum from each quarter of the udder and either the placenta or the uterine fluid, or both, from each animal, whether a ceased reactor or normal, at the time of calving were injected into guinea pigs. In no instance was there any indication of the presence of *Brucella abortus* in the injected material.

At the time that the animals of herd 3 were infected, there were 24 cows that had not previously been exposed to the organism. Two of these were exposed by instilling 3 drops of a virulent culture into the eye of each; each of the remaining 22 individuals was fed 20 cc. of a heavy suspension of the same culture. Of these 24 cows, 6, or 25 percent, became ceased reactors within a period of 24 months after the artificial exposure. One cow (20C in table 1), although proved to have been infected because of the undoubted infection of the guinea pigs injected with the uterine fluid at the calving following the artificial infection, never gave any evidence of a rise in agglutinins following the infection.

DISCUSSION

From the information given in table 1, it will be seen that it was not possible to predict, by the height of the agglutinin titer, whether or not an individual would become a ceased reactor. A summation of the individuals of table 1 which in the three different herds became ceased reactors reveals that, for those whose sera showed complete agglutination at the maximum titer given, there were two with a titer of 1: 400, nine with 1: 200, six with 1: 100, and four with less than 1: 100. Also, there were four animals in herd 1 whose highest titer was 1: 200 or less, as evidenced by a slight trace of agglutination at the titer shown. Furthermore, there were three animals in these different groups which failed to react after the artificial exposure. The animals whose sera never reacted at the prescribed dilution for reactors cannot strictly be classed with the ceased reactors, although in these tests they were included because they might have been carriers of the organism. One such individual (20C in herd 3) definitely was a carrier and therefore a probable spreader. One of the three cows (23A in herd 2) whose sera had never agglutinated the organism, had been in a herd which several years previously had suffered an outbreak of Bang's disease, and thus may have been a ceased reactor when introduced into herd 2.

There can be no doubt of the adequacy of the artificial infection in each of these different herds, since in both herds 2 and 3 more than

half of the cows undergoing their first exposure to *Brucella abortus* aborted their fetuses. In fact, the proportion of aborting cows was higher in both herds 2 and 3 than in herd 1. The details of these infections, and of the response of the ceased reactor cows to second exposure, will be reported elsewhere.

SUMMARY AND CONCLUSIONS

Twenty-one so-called "ceased" reactor cows in 2 herds (14 in 1, 6 in the other) were allowed to commingle with a total of 54 cows from Bang-negative herds through either 1 or 2 gestation periods for each of the normal cows. With the exception of a transitory infection in one-quarter of the udder of 1 of the ceased reactors, *Brucella abortus* was not demonstrated in any of these cows by culture or guinea pig injection at the time of calving. Moreover, none of the 54 normal cows showed evidence of having been infected by any of the ceased reactors, as evidenced by the lack of agglutinins in their sera for *Br. abortus*, except 1 individual whose serum showed a low titer (1: 100) for the organism. This one reactor was in the same herd as the cow showing the transitory infection cited. The writers were unable to demonstrate the organism in this cow either at the time of calving or after slaughter.

The results of these experiments indicate that it is relatively safe to allow ceased reactors to Bang's disease to mingle with noninfected stock. It cannot definitely be said that a ceased reactor cannot be a carrier of *Brucella abortus*, but on the basis of these experiments, it can be stated that the probability is undoubtedly small.

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DIFFERENTIAL INJURY WITHIN VARIETIES, INBRED LINES, AND HYBRIDS OF FIELD CORN CAUSED BY THE CORN EARWORM, HELIOTHIS ARMIGERA (HBN.)[1]

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INTRODUCTION

In the United States the corn earworm (*Heliothis armigera* (Hbn.)) is one of the most destructive of insect pests. Each year it causes severe losses, especially in the Central and Southern States, in tomatoes, soybeans, and cotton, as well as in corn. In field corn the earworm is almost impossible to control at the present time. The small unit value of the crop and the large acreage make the use of insecticides impractical; hence the principal methods of control must consist in cultural practices or biological control. The most promising possibility of success appears to lie in the discovery and use of resistant strains.

The present study was undertaken to determine the range of injury caused by the earworm to various strains and varieties of corn and to classify the varieties, hybrids, and inbreds on the basis of the amount of injury sustained. It was expected that this information would eventually be used in a breeding program directed toward a reduction of the losses from this insect in field corn (*Zea mays* L.)

REVIEW OF LITERATURE

There has been considerable study of the resistance of field and sweet corn to the corn earworm and of the factors associated with this resistance. Much of this study has been devoted to sweet corn because of the greater severity of the injury in sweet corn and the higher unit value of the crop. Until recently, when the problem was partly solved by the use of resistant varieties, this insect was the major factor (*4*)[3] in preventing the profitable growing of sweet corn in the southern part of the United States.

The first detailed study of resistance to corn earworm and one of the first attempts to use plant hybridization in insect control was made by Collins and Kempton (*4*). Their paper presents the most extensive and thorough study so far reported. Some of the facts or probable relationships brought out in this paper are: (1) Resistance to corn earworm may be transmitted from field corn to sweet corn. (2) Low damage was significantly correlated with a number of mor-

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[2] The authors are indebted to R. W. Jugenheimer for permission to use some data collected in 1938, and to various members of the Departments of Agronomy and Entomology for helpful suggestions and criticisms. The following as research assistants were among those who aided in the collection of detailed data: Merle W. Allen, R. O. Snelling, R. G. Dahms, A. L. Robinson, Everett Blood, and Stewart Schell.

[3] Italic numbers in parentheses refer to Literature Cited, p. 99.

phological characters which are mostly interrelated. The greatest correlation was with prolongation of husk, followed by many layers of husk and few husk leaves. (3) Resistant progenies were low both in number of larvae and damage per larva, possibly indicating that plants avoided by moths were also less acceptable to larvae and "that at least a part of the immunity is the result of chemical differences, perhaps the presence of some volatile substance distasteful alike to the moth and the larva." (4) "No difficulty was experienced in securing by hybridization and selection the desired plant characters in combination with the seed characters of sweet corn."

A number of workers have studied, and some have emphasized, the importance of long husks as a protection against corn earworm damage (*2, 3, 5, 6, 8, 9, 11, 15, 16, 18, 19*). Some of these workers also mention the importance of tightness of husk. Phillips and Barber (*16*) studied these characters in considerable detail in 13 varieties. Considering all years and all varieties, the ears with short loose husks had an average of 39.14 kernels injured per ear and those with long tight husks had an average of 18.79, a difference of 20.35 kernels per ear in favor of the latter class. The averages of the other classes were strictly intermediate. Phillips and Barber do not discuss the still greater intervarietal differences in damage shown by their data, which appear to indicate that differences between varieties may be of more importance than difference in husk type (*16, table 3*).

McColloch (*12, 13, 14*) showed that differences in infestation of four varieties could be partly accounted for on the basis of relation of time of silking or stage of maturity to number of eggs. He found that fewer eggs were deposited on plants lacking rough and hairy leaves, and he mentioned the attractiveness of the silks as a character bearing on resistance. This factor has also been studied by Phillips and Barber (*17*). Barber (*1*) studied the relationship between the strong cannibalistic tendencies of corn earworm larvae and the effect of long tight husks in confining the larvae of various instars within a small space where the larger apparently seek out, kill, and feed on the smaller ones. Isely (*10*) has shown that different plants and even parts of the same plant are not of equal value as larval food when measured by size, length of life, or adult fecundity. These and other shorter papers record the following as the chief mechanisms affecting the resistance of corn to corn earworm:

Length of husk, cannibalism of larvae.
Tightness of husks.
Number of husk leaves.
Number of husk layers.
Male florets at tip of ear.
Synchronization of time of silking and peak of oviposition.
Hairiness of plant.
Length of silking period; number of ears per plant.
Total leaf area available for oviposition.
Attractiveness of plant for oviposition.
Value as food for larvae.

Dungan and his associates (*7*) have mentioned certain field corn inbreds that transmit resistance to earworm in Illinois.

MATERIAL AND METHODS

There is reported here information gained at the Kansas station

arlier entomological investigations were conducted by the late 'rof. J. W. McColloch; those since 1928 have been under the direction f the senior author. In Kansas the corn earworm usually has three enerations and a partial fourth, and though frequently these are not istinct, the damage and infestation in corn and other host plants ıcreases more or less uniformly in severity toward the latter part f the season. In most years only an occasional ear of corn escapes ıfestation. The number of ears studied during various years, the ercentage of infestation, and other pertinent data are recorded in ıble 1.

TABLE 1.—*Intensity of corn earworm infestation, Manhattan, Kans., 1929–37*

[All varieties and hybrids]

Year	Ears		Infested	Average class of injury
	Total	Uninfested		
	Number	*Number*	*Percent*	
29	7,058	1,506	78.7	(1)
31	20,159	46	99.9	3.5
32	6,247	623	90.0	2.2
33	19,697	317	98.4	2.4
34 [2]				
35	5,937	66	98.9	3.5
36 [3]				
37	14,484	80	99.4	3.4
Total	73,582	2,638	96.4	3.0

[1] Not used.
[2] Corn killed by drought before ears matured.
[3] Corn too badly damaged by grasshoppers and drought for records

The plant material used has been chiefly that grown in the breeding ıd yield-testing experiments of the Department of Agronomy, supple-ented by a few special experiments. Examination for earworm ımage has usually been made at the time these plots were harvested. ı the earlier years only the percentage of ears infested was recorded. ater it became apparent that even where all ears of two strains were fested they might differ markedly in the average amount of damage ne. Since 1930 the amount of damage on each ear has been deter-ined and the ears from each strain classified into six groups. Class 1 ntained those ears with no evident infestation; class 6, those with e greatest damage. In addition to the direct damage to kernels me weight was also given to poorly filled ears in cases where the ndition of such ears appeared to be due to the cutting of silk by the rn earworm with consequent prevention of fertilization. Where eding was done on the hardened kernels the damage was graded verely. The classification was kept approximately the same from ar to year by the use of a photograph showing characteristic ears in ch class (fig. 1). Later the data gathered in the field were used in lculating the average "class of injury" for each plot.

It is evident that a more detailed record, such as the number of rnels damaged per ear, would have been desirable, but with the nited funds and time available this was not practical. A large plot voted entirely to corn earworm study where the infestation and ımage could have been studied as each strain reached maturity also

would have been desirable. It is known that several possible sources of error were present, particularly the possibility of mistaking the damage of other insects for that of the corn earworm and the difficulty of classifying the nubbins which predominated in various strains during some of the drought years. The necessity for waiting for harvesttime also introduced some sources of error. On the other hand, the refined estimate of damage used, the relatively large samples available in some cases, and the opportunity of obtaining information on the actual strains being used in breeding work, as well as the possibility of studying large numbers of strains, are believed to have outweighed the disadvantages mentioned above. Since the study was largely exploratory the method is believed to have been satisfactory.

The hybrid and inbred material studied has thus not been selected consciously for resistance to earworm, but is rather a more or less

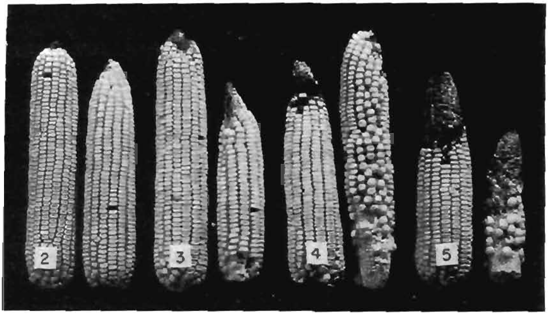

FIGURE 1.—The photograph used in classifying ears for corn earworm damage: Class 1 (not shown), no injury; classes 2 to 5, progressively greater injury; class 6 (not shown), all ears more severely injured.

random sample of the corn germ plasm being used for corn improvement at the Kansas station. A carefully controlled selection for extremes of earworm damage undoubtedly would have produced wider differences even in the absence of detailed information regarding the mechanisms of resistance.

The range of variation found among the different strains studied has sometimes been small, but in those tests in which statistical analysis was possible significant differences have been found to exist. From year to year there has been considerable difference in the average class of injury. Where it has been possible to get information there appears to be a parallel between this measure of damage and the percentage of ears infested.

In 3 years, 1929, 1934, and 1935, a severe early infestation occurred in the agronomy nursery. The feeding of earworms on the opening curl resulted in "ragworm" or "budworm" damage. The percentage of plants damaged was recorded for each strain.

The number of replications studied varied considerably from year to year and in different experiments. Two types of replications are available for study. In many tests the exact strain was repeated from 2 to 10 times and data were secured on each replication. In tests involving single crosses it has been possible to average all strains containing the same parent, thus obtaining replication and a measure of the average performance of an inbred in its hybrids. A similar procedure was followed with double and three-way crosses. Each plot studied usually consisted of 2 rows of 10 hills each so that approximately 40 ears were harvested and rated in regard to class of injury. In the study of the performance of individual inbreds in single crosses the final average was frequently based on several hundred ears. Unless otherwise stated, the term "hybrid" as used in this paper refers to a first generation cross between two inbred lines.

EXPERIMENTAL RESULTS

OPEN-POLLINATED VARIETIES

During the earlier years open-pollinated varieties were the principal objects of study. The infestation and damage to ears of nine of the varieties occurring most frequently are recorded in table 2.

TABLE 2.—*Infestation and damage to ears of open-pollinated varieties at Manhattan, Kans., in certain years from 1925 to 1933* [1]

Variety	Ears infested			1931		1932		1933		Average ear infestation	Pride of Saline ear infestation in same tests	Average class of injury 1931 to 1933
	1925	1927	1929	Ears infested	Average class of injury	Ears infested	Average class of injury	Ears infested	Average class of injury			
	Percent	*Percent*	*Percent*	*Percent*		*Percent*		*Percent*		*Percent*	*Percent*	
Hays Golden	-----	64.3	68.3	100	2.98	88.3	2.03	95.4	2.16	83.3	88.5	2.39
Reid Yellow Dent	58.4	55.4	76.1	100	3.10	85.6	1.91	97.1	2.17	78.8	86.8	2.39
Harmon White	----	-----	84.9	100	3.29	90.7	2.11	100.0	2.22	93.9	95.1	2.54
Freed White	69.8	62.0	72.0	100	3.23	86.1	2.14	93.4	2.28	80.9	86.8	2.55
Cassel White	66.2	72.0	66.1	100	3.00	88.7	2.12	100.0	2.66	82.3	86.8	2.59
Colby Bloody Butcher	64.5	69.9	57.6	100	3.29	90.4	2.09	100.0	2.44	80.4	86.8	2.61
Pride of Saline	77.1	63.2	84.5	100	3.41	95.9	2.22	100.0	2.33	86.8	-----	2.65
Midland Yellow Dent	77.3	44.1	86.1	100	3.68	88.1	2.15	96.4	2.27	82.0	86.8	2.70
Boone County White	------	57.2	76.8	100	4.62	94.4	2.29	-------	----	82.1	85.7	[2] 3.46

[1] Arranged in order of average class of injury.
[2] 2-year average.

Studied were made of these varieties in 1925, 1927, 1929, 1931, 1932, and 1933. In order to provide a convenient means of evaluating the data, the average infestation of each variety has been compared with the average of Pride of Saline in the same tests. This method must be used with some caution since the relative infestation in some cases varied with the year and severity of infestation. Strictly speaking, only those tests conducted in the same season are comparable. In interpreting the data, no serious errors will be made if this fact is kept in mind.

It is evident that the infestation on all varieties was much heavier during the last 3 years than during the first 3. The average percentage of ears infested for the 6 years indicates that Pride of Saline had the highest infestation of any variety represented in all 6 years, but the

the variety with the lowest infestation, is probably of no great importance.

The most obvious relationship between infestation or damage is that with stage of maturity, particularly with the date at which flowering or silking takes place. During the earlier years this maturity relationship was measured in terms of the days from planting until the time when one-tenth or one-eighth of the plants in a given plot were shedding pollen. Pollen shedding has been shown to be very highly correlated with the appearance of silks, and hence with the availability of silks, which are the preferred place of oviposition for the corn earworm moths. In the study of scatter diagrams of the data gathered during the several years there appeared to be a direct relationship between time of flowering as measured by pollen shedding and both percentage of infestation and class of injury. In 1929, when the largest number of varieties were available for study, the correlation between date of one-tenth pollen shedding and percentage of ears infested was $r=0.54$ for the 45 entries. This correlation is considered highly significant.[4] The data for the years 1931 and 1932, in which 37 and 26 entries, respectively, were studied, show a similar relationship between the time of pollen shedding and the average class of injury. In the other years somewhat fewer varieties were available for study.

From these data, obtained with open-pollinated varieties and gathered under the severe conditions of infestation in Kansas, there appears to be no very dependable difference of practical importance that can be measured independently of the date of pollen shedding or silking. This does not necessarily mean that there are no real differences be- between the different varieties. It seems reasonable to assume that the great variability within each variety obscures any real differences that might be demonstrated under lower infestation and with larger samples, since susceptible strains within the varieties would render them especially sensitive to the synchronization of time of silking with the peak of moth oviposition. The belief that inbred strains and their hybrids would be more favorable material for study, and the gradual increase of interest in corn hybrids, led to the abandonment of further study of varieties. In recent years emphasis has been placed on the various commercial and experimental hybrids.

INBREDS AND HYBRIDS WITH REFERENCE TO EAR INJURY

Relation of Class of Ear Injury to Stage of Flowering

Since 1930 many different hybrids in the agronomy testing nursery have been studied, but only a part of the data are here reported. These hybrids consist of single crosses, three-way and four-way crosses, and top crosses. Most of the data presented below concern single crosses since these tend to be more uniform and to give a better measure of the performance of the respective inbreds. The ability of an inbred to increase the resistance in the hybrids in which it occurs is the primary object of interest in these studies. Much of the information gained from hybrids other than single crosses has been used along with other data in selecting the more desirable combinations for the practical corn-breeding project.

Since the hybrids studied have depended on the time and material available, the number of replications and the number of hybrids

involved have varied greatly from year to year. Practically all of the hybrids studied have come from the breeding and testing nursery and from a few small plots planted especially for the study of corn earworm infestation. In the agronomy nursery the hybrids were usually grouped on the basis of similarity of origin or time of maturity. In the various tests in which replications and arrangement of plots permitted the use of statistical methods, a minimum difference of from

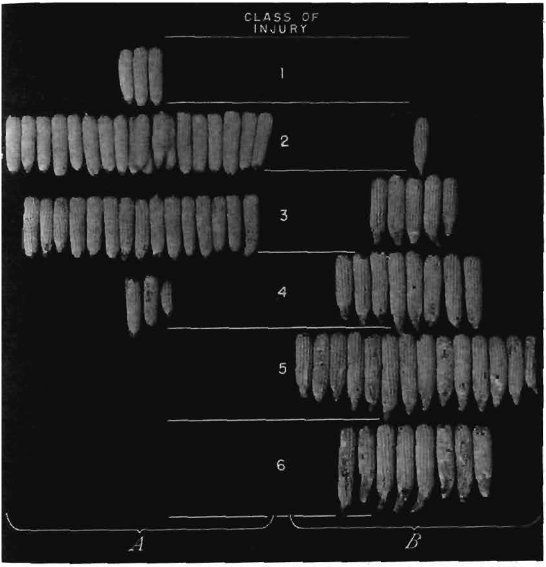

FIGURE 2.—All corn produced in 1938 by plots of two single-cross hybrids graded according to class of injury and showing contrasting earworm injury. The plots were located on opposite sides of a 3-foot alley: *A*, Row 16, Ks. Hy. 126 × Ks. Y. S. 151, average class of injury 2.45, three uninfested ears; *B*, row 103, Ks. M. 201 × Ind. 38–11, average class of injury 4.64, frequent damage far down on ear, 3 days later in flowering than row 16. Numerals on figure indicate class of injury.

0.5 to 1.0 class of injury has been found to be significant. The consistent difference between the hybrids with light injury and those with severe injury, as shown in figures 2 and 3, is also certainly of economic importance.

In all of the years the date of flowering, as represented by the appearance of silks or the shedding of pollen, has been available for com-

parison with the amount of earworm injury. Pollen shedding and silking are known to be highly correlated, and both characters have been used as a measure of the relation between time of silking and amount of damage, thereby giving some idea of the importance in various strains of the synchronization of time of silking and peak of moth oviposition. In 1931 the extension of the husk beyond the ear tip was determined for each of 20,159 ears in a study of the effect of this variable on earworm damage.

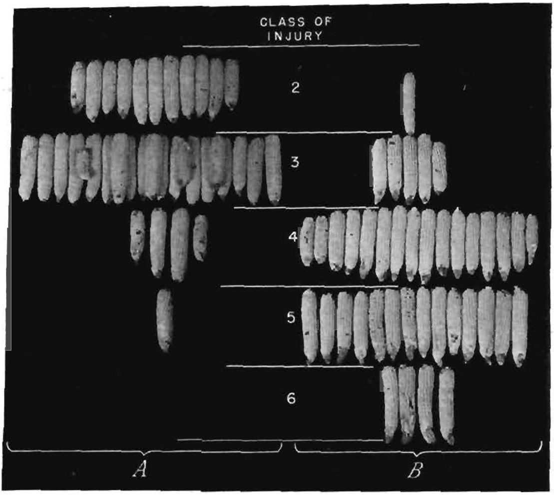

FIGURE 3.—Two single-cross hybrids graded according to class of injury and showing contrast in earworm injury. Both hybrids have all ears injured and have one parent in common: *A*, Row 123, Ks. Hy. 126 × Ks. Y. S. 175, average class of injury 2.86; *B*, row 121, Ks. Hy. 126 × Ks. Y. S. 167, average class of injury 4.38, severe injury to hardened kernels does not show well in the photograph; 1 day earlier in flowering than row 123. Numerals on figure indicate class of injury.

In a group of 100 early single crosses in 1931 there was a range of average class of injury from 2.70 to 4.61. Pollen shedding took place in this experiment between the dates July 4 and 17. A study of the correlation between the average class of injury and the days from planting until one-eighth of the plants in the respective plots were shedding pollen, appeared to show no relationship ($r=-0.05$). A study of the scatter diagram and of records of the hybrids with the highest and lowest average class of injury (table 3) suggests the slight possibility of a curvilinear relationship between these two characters.

ABLE 3.—*Husk extension, time of flowering, and class of earworm injury, in 12 slightly and 12 heavily injured hybrids from a group of 100 early single crosses and of 149 Pride of Saline single crosses studied at Manhattan, Kans., 1931*

100 EARLY SINGLE CROSSES

ow o.	Hybrid	Husk extension: Average	Husk extension: High	Husk extension: Low	Open husks	Ears showing indicated class of injury: 1	2	3	4	5	6	Total ears	Average class of injury	Time from planting to ½ pollen shedding
		Centimeters	*Centimeters*	*Centimeters*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*		*Days*
59	17 × 16	+3.40	7	-1		0	7	16	0	0	0	23	2.70	62
105	36 × 22	+1.55	5	−5	1	0	8	26	1	0	0	35	2.80	63
39	12 × 22	+2.55	3	−3	1	0	15	14	8	0	0	37	2.81	62
25	9 × 11	−.85	5	−5	1	7	14	8	21	0	0	50	2.86	59
91	29 × 22	+4.65	6	2		0	12	21	7	0	0	40	2.88	61
43	13 × 26	+3.60	6	1		0	6	27	2	0	0	35	2.89	59
37	12 × 13	+3.70	7	0		0	9	24	3	1	0	37	2.89	58
98	34 × 35	+2.90	6	0		0	13	24	8	0	0	45	2.89	50
33	10 × 35	+2.10	6	−4		0	12	30	7	0	0	49	2.90	60
45	13 × 14	+4.05	6	2		0	6	33	3	0	0	42	2.93	58
72	22 × 37	+.80	5	−4		0	16	23	7	0	2	48	2.94	62
69	20 × 27	−1.40	6	−6		0	5	18	2	1	0	26	2.96	61
	Average	+2.25	5.8	−2									2.87	59.6
	Freed check [1]		14	−8									3.60	
87	27 × 26	+4.20	8	0		0	0	4	17	10	0	31	4.19	60
106	37 × 23	−.25	5	−4		0	1	18	6	2	13	40	4.20	64
93	32 × 35	+3.10	5	1		0	0	4	24	15	0	43	4.25	62
7	5 × 30	−3.10	7	−7	5	0	0	5	25	13	2	45	4.27	55
1	1 × 13	+1.15	5	−5	2	0	2	7	15	8	7	39	4.28	55
75	23 × 22	−.30	6	−5		0	3	9	10	3	12	37	4.32	64
3	5 × 1	−1.95	9	−7	11	1	2	8	13	20	6	50	4.37	53
5	5 × 14	+.50	3	−2		0	0	4	22	12	4	42	4.38	57
94	33 × 37	+2.95	6	−5		0	0	6	21	7	0	34	4.39	62
51	15 × 23	−2.40	3	−5		0	0	6	17	15	4	42	4.40	62
74	23 × 18	−2.25	6	−6		0	1	9	12	4	13	39	4.49	64
73	23 × 8628	−4.60	−1	−7		0	0	8	16	15	12	51	4.61	65
	Average	−.24	5.2	−4.3									4.35	60.3

[1] 12 rows.

TABLE 3.—*Husk extension, time of flowering, and class of earworm injury, in 12 slightly and 12 heavily injured hybrids from a group of 100 early single crosses and of 149 Pride of Saline single crosses studied at Manhattan, Kans., 1931*—Continued

100 EARLY SINGLE CROSSES—Continued

Row No.	Hybrid	Husk extension: Average	Husk extension: High	Husk extension: Low	Open husks	Ears showing indicated class of injury: 1	2	3	4	5	6	Total ears	Average class of injury	Time from planting to ½ pollen shedding
		Centimeters	*Centimeters*	*Centimeters*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*		*Days*
8	5 × 14	+6.65	11	1		0	21	12	0	0	0	33	2.36	68
98	41 × 53	+4.40	13	−2		0	15	15	0	0	0	30	2.50	64
28	10 × 17	+2.30	4	−3		0	9	25	0	0	0	34	2.71	64
11	5 × 19	+2.10	5	−1		0	9	26	0	0	0	35	2.71	67
78	27 × 34	+10.45	13	6		0	8	21	0	0	0	32	2.75	69
23	9 × 12	+5.45	9	3		0	4	21	0	0	0	28	2.86	67
2	2 × 11	+3.40	7	1		0	12	23	7	0	0	42	2.88	65
44	48 × 56	+5.60	9	3		0	9	10	6	0	0	25	2.88	68
18	8 × 14	+3.85	8	−1		0	8	28	4	0	0	40	2.90	66
9	5 × 17	+5.10	8	4		0	5	26	2	0	0	33	2.91	65
16	7 × 18	+9.10	13	6		0	3	36	0	0	0	39	2.92	66
41	48 × 52	+5.95	11	3		0	6	15	4	0	0	25	2.92	66
	Average	5.36	9.25	1.9									2.78	66.3
	Pride of Saline checks [2]													
35	47 × 57	+4.60	9	0		0	0	12	12	1	10	35	3.70	65
24	45 × 52	+8.35	13	7		0	0	5	16	16	0	37	4.26	67
75	26 × 39	−1.75	1	−4	12	0	0	2	21	15	0	41	4.30	69
56	50 × 55	+4.65	7	−3	2	0	0	9	8	5	8	30	4.32	66
31	47 × 53	+2.30	7	−2	6	0	0	4	22	28	0	54	4.40	65
87	29 × 32	+3.25	9	−3		0	0	0	28	25	0	53	4.44	71
46	48 × 58	+4.75	7	2		0	0	7	8	12	7	34	4.47	64
52	49 × 56	+9.25	12	6		0	0	10	14	1	18	43	4.56	68
36	47 × 58	+6.70	15	0		0	0	5	7	10	9	31	4.63	65
49	49 × 51	+3.50	8	−7	2	0	0	7	15	9	18	49	4.71	65
51	49 × 55	+6.55	11	4		0	0	3	5	5	11	24	4.78	67
53	49 × 57	+.15	5	−5		0	0	3	5	42	14	64	5.00	68
	Average	4.36	8.7	−0.4									4.58	66.7

[2] 9 rows.

The number of hybrids at the two ends of the range of maturity is not sufficient to indicate whether this is actually the case. Such a curvilinear relationship, if it exists, could be due perhaps to the approach to a peak of moth oviposition at the beginning and another at the end of the range of flowering. An examination of table 3 will indicate that it is possible to pick out hybrids each with low damage but with a considerable difference in the dates of flowering. Thus hybrids with the lowest and third from the lowest average class of injury were evidently of approximately the same stage of maturity as were the hybrids with the third and fourth from the highest average class of injury.

In 1931 a study was also made of 149 hybrids involving more than 50 different inbreds selected from Pride of Saline. These showed a range in average class of injury from 2.36 to 5.05 and a range in maturity as measured by days from planting to one-eighth pollen shedding of from 63 to 74 days. A study of the scatter diagram of this group of hybrids indicates little more relationship between maturity and average class of injury than was shown by the early hybrids mentioned above, nor is there any evidence of curvilinear relationships. The data from the 12 highest and 12 lowest in earworm injury are shown in table 3. A study of the hybrids with the lowest and the highest average class of injury gives further evidence of the lack of relationship between these two variables. For instance, the hybrid with the highest and the one with the lowest average class of injury were identical in time of shedding pollen and probably in time of silk emergence.

Among these Pride of Saline hybrids the relation between class of injury and the days to pollen shedding was studied in the 51 constituent inbreds, the data being based on the average performance of inbreds in from 2 to 10 single crosses. The average days from planting to pollen shedding varied from 64.0 to 72.8. On this basis of performance of inbreds in crosses there again appeared to be no close relationship between average class of injury and days to one-eighth pollen shedding. This is further indicated in table 4, in which the parental strains were divided into quartiles and the average of the two variables for the different quartiles given.

TABLE 4.—*Relation of class of injury by corn earworm to (1) days from planting to one-eighth pollen shedding and (2) to husk extension, among 51 Pride of Saline inbreds, lines based on performance in single crosses*

Quartile No.	Parental strains	Average class of injury [1]	Average time from planting to ⅛ pollen shedding	Average husk extension
	Number		*Days*	*Centimeters*
1	13	3.12	66.4	3.57
2	13	3.49	67.3	4.41
3	12	3.73	68.8	3.40
4	13	4.07	67.7	3.61

[1] Arranged in order of average class of injury.

There is a difference of only 1.3 days between the quartile with the lowest class of injury and that with the highest. The third quartile,

moreover, averaged later than the fourth in the date of flowering, indicating no clear-cut trend between these two variables.

This same lack of any obligate relationship between stage of maturity, as measured by pollen shedding or silking, and class of injury has also been found in most of the other years in which it has been studied in sufficient detail. In 1932, in a special test involving some of the Pride of Saline hybrids, the average class of injury in individual hybrids ranged from 1.80 to 3.18 in two replications. The 2 individual hybrids at the extremes of the range of class of injury varied only by 0.5 day in the number of days from planting to one-eighth pollen shedding. All 20 hybrids involved taken as a group, however, show a slight tendency for the later flowering ones to have a higher average class of injury. When the inbreds in this same test were studied on the basis of their performance in single hybrids there appeared to be no close relationship between this stage of maturity and class of injury. For instance, inbred parent 14, represented in 10 hybrids, and inbred parent 30, represented in 8 hybrids, showed a difference in class of injury of 0.52 ± 0.1004, which is statistically highly significant. These same parents as measured by their performance in hybrids differed by 1.3 days in respect to this stage of maturity. This small difference in dates of flowering can hardly be considered to be of biological significance.

In 1937, 35 three- and four-way crosses and top crosses varied in class of injury from 3.04 to 3.95 as an average of four replications for each hybrid involved. An analysis of variance indicates that the difference in earworm injury between the hybrids is significant. These hybrids ranged in average days from planting to one-eighth pollen shedding from 69 to 77 days, and in days from planting until one half the plants were in silk, from 74 to 84 days. A scatter diagram shows a relationship between both these variables and the average class of injury. Those hybrids with the earlier pollen shedding or silking tend to have the higher class of injury. In one experiment concerned with 10 of these hybrids the correlations of time of flowering with class of injury were significant (with days from planting to one-eighth pollen shedding, $r = -0.69$; with days to one-half silking, $r = -0.68$). In another experiment in which there were 20 hybrids the correlations were still negative but not significant (with days from planting to one-eighth shedding, $r = -0.29$; with days to one-half silking, $r = -0.049$). This trend is exactly the reverse of that found in most years, and, incidently, is the reverse of that found in a group of popcorn varieties which were studied the same year and which were silking and shedding pollen at approximately the same time. In spite of this reversal of the relationship between class of injury and stage of ear maturity, certain hybrids which had shown a low average class of injury in other years also showed a low average class of injury in 1937. Thus the resistant strains do not appear to be as greatly influenced by date of silking as the susceptible ones. This fact would indicate that it may be the susceptible individuals within a varietal population, or the susceptible strains within a group of hybrids, that are primarily responsible for any general relationship that may exist between injury by earworm and the period of flowering.

Relation of Class Ear Injury to Length of Husk Extension

The length of the husk extension beyond the ear tip was studied on the group of early single crosses in 1931, as shown in table 3. Each hybrid appeared to be reasonably uniform for length of husk, although marked differences in husk covering were evident between hybrids. There was a maximum difference of 12 cm. between the average of hybrids with the least husk extension and those with the greatest. Negative readings indicate ears whose tips protruded beyond the ends of the husks. The correlation between the average class of injury and the average husk extension was negative and highly significant statistically ($r=-0.29$). A study of those hybrids having the least and those having the most injury (table 3) shows that there are 2 among the 12 hybrids with the lowest average class of injury in which the tip of the ear extends beyond the husk, on the average, and that some of the hybrids in the group with the highest class of injury have husks which are as long as or longer than the hybrid with the lowest amount of damage. This would appear to indicate that while the length of husk is a factor among some hybrids it is not the only factor involved and in the case of some hybrids is perhaps of negligible importance.

The same year (1931) the husk extension was measured on the 149 Pride of Saline hybrids listed in part in table 3. In most of these hybrids the husk extended beyond the ear. There was a range in husk extension from an average of +11.15 cm. to −3.0 cm. among the different hybrids represented. In this case there appears to be some relationship between husk extension and class of injury, but this certainly is not an obligate relationship since the average husk extension of the hybrid with the least injury was +6.65 and that of the hybrid with the second highest class of injury was +6.55. Similar comparisons were possible among the other hybrids. When these hybrids are studied on the basis of the constituent inbreds as summarized in table 4 there is only 0.04 cm. difference between the average length of husk extension of the 13 parental strains with the lowest class of injury and that of the 13 parental strains with the highest class of injury. Detailed measurements were not taken in succeeding years in view of the apparent small obligate importance of the character of husk extension within the range available for study among the hybrids here.

Performance of Inbreds in Hybrid Combination Under Various Seasonal Conditions as Measured by Class of Ear Injury

A group of Pride of Saline hybrids has been studied through a period of years and the results have been analyzed on the basis of constituent inbreds. A summary of this analysis is shown in table 5.

TABLE 5.—*Corn earworm injury rating*[1] *of inbred lines from Pride of Saline based on their performance in single crosses, 1930–38*

Inbred No.	1930			1931			1932			1933			1938			3-year average class of injury	4-year average class of injury	Total replications 1931–38
	Plants infested	Rank	Hybrids	Average class of injury	Rank	Hybrids	Average class of injury	Rank	Hybrids	Average class of injury	Rank	Hybrids	Average class of injury	Rank	Hybrids			
	Percent		*Number*			*Number*			*Number*			*Number*			*Number*			*Number*
[illegible]	85.3	2	6	2.82	1	5	2.12	2	6				2.52	3	5	2.49		16
[illegible]	88.3	4	8	3.28	8	2	2.16	4	2	2.19	8	1	2.19	1	1	2.51	2.46	6
[illegible]	91.9	7	3	3.08	5	3				2.15	4	2	2.40	2	4	2.54		9
[illegible]	79.6	1	8	2.99	2	6	2.15	3	10	2.21	9	5	2.68	4	8	2.61	2.51	29
[illegible]	89.1	5	7	3.01	4	5	2.18	5	4	2.14	3	1	3.01	7	3	2.72	2.59	13
[illegible]	93.3	8	8	3.00	3	3				2.29	10	2	2.93	6	7	2.74		12
[illegible]	98.2	13	7	3.71	14	4	2.43	9	11	2.15	4	2				2.76		17
[illegible]	87.0	3	5	3.16	6	3	2.07	1	4				2.86	5	4	2.79		11
[illegible]	100.0	18	10	3.46	10	9	2.43	8	8	2.59	13	2				2.82		19
[illegible]	96.7	10	9	3.49	12	9				2.09	2	1	3.21	11	5	2.93		15
[illegible]	100.0	18	2	4.14	19	2	2.66	10	8	2.00	1	1				2.93		11
[illegible]	95.1	9	4	3.27	7	3	2.36	7	16	2.90	16	1	3.21	11	4	2.94	2.93	24
[illegible]	89.9	6	9	3.42	9	7				2.16	7	2	3.29	13	8	2.96		17
[illegible]	98.5	15	6	3.69	13	4	2.33	6	7	2.88	15	2				2.97		13
[illegible]	97.8	11	6	3.84	16	6				2.37	12	1	3.02	8	7	3.08		14
[illegible]	98.4	14	8	3.46	10	8				2.78	14	2	3.08	9	4	3.11		14
[illegible]	99.1	17	8	3.85	17	6				2.15	4	4	3.60	15	2	3.20		12
[illegible]ide of Saline	90.5			3.70		9				2.41		9	3.54		1	3.22		19
[illegible]	98.5	15	9	3.73	15	8				2.90	16	1	3.13	10	8	3.25		17
[illegible]	97.9	12	10	4.10	18	10				2.34	11	4	3.32	14	17	3.25		31

[1] In rating injury, 1 denotes ears with no injury.

A study of these hybrids was begun in 1930, in which year only the percentage of plants infested was recorded; in later years damage was recorded on the basis of class of injury. This is the same group that has furnished much of the information given above. In 1930 and 1931 records were taken on the hybrids made up from a group of 51 inbreds. Notwithstanding the fact that the 1930 data were taken on percentage of ears injured and the 1931 data on average class of injury, there is a highly significant interannual correlation between the performance of these 51 inbreds ($r=+0.53$). Twenty-three of these inbreds were represented by hybrids in 1938 and there is again a highly significant interannual correlation in the performance of the two groups ($r=+0.75$).

In table 5, 19 of these inbreds which were represented by hybrids recorded in 4 or more years are ranked on the basis of a 3-year average class of injury. The 3-year average is made up of from 5 to 29 hybrids and each average is based on from 200 to more than 1,000 ears. The average must be studied in the light of the number of hybrids involved in the different years. Inbreds 50 and 48 and probably 30 have too high a rating in table 5 because of the particular years and the number of replications involved in the average. For the other inbreds the ranking is relatively similar in the different tests despite wide seasonal differences and differences in the method of recording injury in one of the years. It appears, therefore, that the differences represented in the table by those inbreds with high and low class of injury are genetic and hence of importance in a breeding program. Corroborative evidence has been furnished by the use of a certain single cross in hybrids involving resistant inbreds in double and three-way crosses. Single cross 4 × 14 proved to be especially useful. During the course of the experimental work a number of inbreds have been discarded, particularly those transmitting extreme susceptibility to corn earworm and other undesirable traits. Hence, it is probable that in the later years the spread between susceptible and resistant hybrids would have been greater had more of the susceptible types been retained.

INBREDS AND HYBRIDS WITH REFERENCE TO BUDWORM INJURY

Each year the corn ear worm does some damage at Manhattan to the upper leaves of young corn plants. This type of injury to the curl has been known as budworm or ragworm injury. On three occasions the damage in the breeding and testing nursery has been so severe that certain rows in which no plants were injured were very conspicuous. This called attention to the possibility that susceptibility to this type of damage might be inherited. In 1929 records were taken, and a range up to 50 percent of the injured plants was found. Since this type of damage does not occur with equal intensity each year there was no opportunity to restudy the strains on which records were taken. In 1934 a heavy budworm infestation occurred, and records were taken on most of the hybrids and inbreds in the agronomy nursery. One group of single-cross hybrids being tested for the first time was of especial interest on account of the wide range in the amount of damage in various plots. All of the corn in these nurseries was destroyed by drought and most of it cut early for forage; hence the same hybrids were replanted the following year with but little change in the number of strains represented. Thus in 1935 when a second budworm in-

festation of similar intensity occurred it was possible to get a second record on identical material. The plants in the several strains were fairly closely related and did not differ materially in height at the time of the infestation. At harvesttime in 1935 it was possible to take records on the class of injury of ears from the same plants previously recorded for budworm injury. The injury to ears, however, showed wide differences according to location in the field, and it was necessary to correct the figures for place effect on the basis of uniform checks which occurred every 10 rows. The early spring infestation in the curl of the plant showed no such variability. The performance of each inbred was calculated on the basis of the performance in hybrids in which it occurred, and the data were averaged for percentage of plants infested in the bud in respective years and average class of ears injured. In 1934, data were taken on the percentage of plants showing curl injury among the actual inbred parents. The number of plants concerned in this figure varied from 16 to 135. A summary of the results obtained is given in table 6.

Table 6.—*Relation of curl injury in inbred lines and hybrids to injury of ears in hybrids by the corn earworm—New Yellow hybrids, 1934–35*

Inbred line No.	Inbred line		Performance in hybrids 1934		Performance in hybrids 1935			
	Plants	Infested	Crosses represented	Average plants infested	Crosses represented	Average plants infested—curl injury	Average class of ear injury: Observed	Average class of ear injury: Corrected for place effect
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*		
63	60	14	9	5	9	11	3.8	3.6
57	22	0	12	5	12	23	3.3	3.1
25	46	7	6	9	6	21	4.1	3.9
33	31	0	10	11	11	15	3.7	3.5
48	109	3	14	11	14	17	2.8	3.1
51	48	5	27	11	27	18	3.3	3.3
67	34	34	14	12	14	20	3.4	3.6
30	16	31	13	12	13	20	4.0	3.6
53	32	12	21	13	20	19	3.7	3.7
60	84	24	7	13	7	19	3.4	3.2
68	84	19	6	13	6	25	3.5	3.9
70	31	4	5	14	4	18	4.2	3.8
54	31	18	7	14	7	23	4.1	3.9
66	82	55	10	15	10	27	3.7	3.6
45	74	38	8	15	8	35	2.9	3.4
26	76	6	19	18	18	24	3.5	3.4
55	65	52	12	23	11	36	3.1	3.2
56	58	22	24	21	24	37	3.6	3.4
75	45	70	18	24	17	24	3.9	3.8
58	33	17	8	25	7	33	3.3	3.3
64	16	75	5	26	5	37	3.7	3.8
61	135	66	15	27	15	32	3.3	3.3
28	52	25	7	29	7	31	3.2	3.5
73	45	18	4	33	4	37	4.2	3.9
59	56	45	8	35	8	31	3.2	3.2
50	43	68	9	50	9	40	3.8	3.8
42	18	73	9	50	9	30	2.5	3.3

An examination was made of the relationship between the percentage of inbred plants infested and the average percentage of plants infested among the hybrids in which each inbred was represented. The correlation between the curl injury to parents and to F_1 hybrids in 1934 was highly significant ($r = +0.67$). The interannual correlation between the percentage of infested plants in identical hybrids in

1934 and 1935 was also highly significant ($r=+0.73$). On the basis of performance of inbreds it was possible to compare the percentage of plants showing budworm injury with the average class of ears injured in 1935 on hybrids from these same inbred lines. Among hybrids representing 27 inbred lines the correlation between these two kinds of injury was not significant ($r=+0.003$).

These data indicate that resistance to budworm injury, whatever its cause, is inherited, and in some cases, at least behaves as a dominant character. The lack of close relationship between average class of ear injury and percentage of plants showing budworm injury indicates either that these two characteristics are governed by different sets of genetic factors or that the difference in ear injury which results from difference in resistance was distorted so by the date of silking that relationship to budworn injury is obscured. In view of the results reported elsewhere in this paper on class of ear injury, it is believed that the former explanation is the correct one. An inbred which confers on it hybrids a high degree of resistance to budworm injury would be desirable since such a character might tend to limit the increase of the earworm in the field.

MASS SELECTION FOR EARWORM RESISTANCE

In 1932 a beginning was made in a mass-selection experiment for altering earworm resistance within the open-pollinated variety Pride of Saline. Two lots of seed selected in that year, consisting of a group of uninfested ears and a group of fairly severely injured ears, were planted in neighboring areas on the agronomy farm in 1933. Five samples of approximately 50 ears each from consecutive stalks were selected the following fall from distributed areas within each plot. The ears from the uninfested seed corn averaged 0.2 of a class lower than the ears from the infested seed corn. This difference probably is not statistically significant, but an examination of the data indicates that the average of every sample from the plot with infested seed corn showed more injury than the average of the entire other group, and four of the five samples from the plot in which the seed corn was free from infestation showed less injury than the average of the other group. Hence, in this first year the mass selection had given slight but apparently consistent improvement in the expected direction. Ears similar to those selected the preceding year were picked from the two plots and planted in 1934. That year both plots were entirely destroyed by drought and the seed supply was lost. It appears, however, that mass selection may not only provide a promising method for improving open-pollinated varieties for insect resistance, but may furnish a source of breeding material for earworm-resistant inbreds.

DISCUSSION AND CONCLUSIONS

In the material studied, the relationship between date of flowering and earworm damage to ears was much more apparent among varieties than among inbreds and hybrids. Under conditions of extreme infestation such as occur at Manhattan, this relationship is probably due to the greater variability in individual plants of open-pollinated varieties which tends to obscure any resistance that may be present in the general population. The influence of date of flowering on ear-

worm injury would be most evident in those strains or individua plants that lack other mechanisms of resistance, such as those listed earlier in the paper. It is the predominance of these more susceptibl individuals within a variety which is probably responsible for the relationship mentioned. Under other environments with lower inci dence of the corn earworm it is possible that the longer silking perio of varieties as compared with hybrids would place varieties at a dis advantage on the basis of percentage of infestation.

Among the hybrids or inbreds studied there have been varyin numbers of the more susceptible types in the different experiments and this fact would account for a part of the inconsistency in the cor relation between injury and time of flowering in the various exper mental groups. An examination of table 3 will furnish addition information on some of these points. In the experiments there re corded, as in other experiments, the hybrids having a low class of injury are generally less variable in respect to this character than a hybrids with a higher class of injury. The ears of the less-injure strains are classified most frequently in two or three classes, where those of the more severely injured strains fall into four or five classe less commonly into three or six. The greater variability of the latt hybrids is perhaps a result of their sensitiveness to environment influences because of higher susceptibility. These conclusions a based on conditions of high infestation at Manhattan and might n hold under different conditions or in tests where damage was record as a percentage of infested ears.

Another evident reason lies in the seasonal variability in the pea of moth oviposition. Unfortunately no detailed information available on the seasonal life history of the insect during the years which these observations were made. The years concerned ha differed greatly in climatic factors, and unquestionably these have h a differential effect on insect and plant life histories.

The length of the husk extension would have a greater influence earworm damage among strains which are susceptible in otl respects than among those which are more resistant. This m explain the correlation between husk extension and ear damage in so cases, but at the same time it is equally evident that long husks not responsible for low damage in some of the hybrids. It appe possible, therefore, to obtain some degree of resistance to earwc injury independent of both husk extension and period of floweri From time to time observations have been made on tightness of h and other characters which might be concerned in earworm inj So far no promising or easily measured characters have been fou

The most important result of these investigations is the evide obtained that certain inbreds as represented by their performanc hybrids are consistently less injured than others. Year after y in spite of considerable differences in seasons and in intensity of festation, there appears to be evidence of the inheritance of resist independent of some of the measurable mechanisms and of fluctuat caused by the environment. The differences in earworm dam although small, may be of importance in their promise of future p bilities in specific selection for earworm resistance. The hyb studied have been from inbreds selected for characters other t earworm resistance. Such resistance as has been found has resu from random selection insofar as earworm damage is concer

ince differences exist under these circumstances it is probable that onscious selection for earworm resistance would yield greater differnces. It appears worth while to utilize such differences as are at resent available in commercially desirable hybrids. Corn breeders hould not lose sight of the possibility of including in their inbreds and ybrids the best qualities of insect resistance available in the germ lasm of the foundation varieties.

SUMMARY

A consistent tendency toward resistance or susceptibility to ear-orm damage to ears has been transmitted by certain inbred lines of orn.

Length of husk extension and date of flowering have some influence n the amount of damage to ears, particularly in heterogeneous maerial, but many marked breaks in the correlations occur, suggesting ther and more subtle causes of differences in severity of injury.

Under conditions at Manhattan, Kans., the relatively susceptible trains appear more sensitive to the influence of date of flowering and ength of husk extension than do the relatively resistant strains. The reater influence of these factors in open-pollinated varieties may be ue to the high proportion of susceptible individuals present in the aterial studied.

Differences in resistance and susceptibility to injury to the developg curl or bud of young corn plants are also inherited. Such differnces appear to be independent of the differences in resistance to damto ears caused by the same insect.

here are indications that resistance to earworm injury may be eased by mass selection within an open-pollinated variety.

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PHOTOTHERMAL INDUCTION OF FLOWERING IN SUGAR BEETS[1]

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INTRODUCTION

Induction of flowering in sugar beets (*Beta vulgaris* L.) has two nportant practical aspects. Development of seedstalks, called bolt- g, is objectionable when the crop is grown for sugar production, but hen the crop is grown for seed production initiation and complete ·velopment of seedstalks in all the plants is desirable. The first ırly top-resistant varieties were easy-bolting types (*4*).[3] Their)lting tendency was sometimes objectionable in plantings for sugar, ıt it was beneficial in plantings for seed. Therefore seed of these ırieties could be produced abundantly in relatively mild climates by anting in early fall and allowing the plants to grow in the field ırough the winter (*21*). Breeding to combine nonbolting with curly ·p resistance was started as soon as it became evident that there were)jections to the easy-bolting tendency of the first curly top-resistant ırieties. Nonbolting varieties with curly top resistance were ·omptly developed; but, when the effort was made to produce seed r the method of overwintering in the field in the relatively mild ımates where beet-seed growing had become established, these vari- ies failed to reproduce satisfactorily (*22*). Nonbolting varieties ·ed in northern Europe gave similar results.

These experiences emphasized the need for fuller knowledge of the ·inciples concerned in bolting and sexual reproduction. This paper ·esents results of studies of some of the physiological and genetic pects of the problem.

REVIEW OF LITERATURE AND DEFINITION OF TERMS USED

In much that has been written about causes of flowering, the combined effect of temperature and day length, or photoperiod, which is required by some plants, has not been considered. This is especially true in much of the literature on photoperiodism that has appeared since the classic discovery of Garner and Allard (*9*). The specific effect of temperature has been given prominent attention, however, in extensive Russian work that began with the treatment of seed to induce flowering. The action of temperature has been referred to (*12*) as thermal induction and the influence of day length as photoperiodic induction. Chroboczek (*5*) and Steinberg and Garner (*24*) showed that both low-temperature treatment and long photoperiods are

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[2] The Curly Top Resistance Breeding Committee cooperated in the work in northern Utah and southern Idaho and in the bolting test at Riverside, Calif. This committee represents all the beet-sugar companies in the Western States. Land was furnished for the bolting test at Riverside by the California Agricultural Experiment Station.

[3] Italic numbers in parentheses refer to Literature Cited, p. 123.

favorable to flowering in beets. Roberts and Struckmeyer (*23*) reported important associations between temperature and photoperiod in relation to flowering in a number of species, including beets. The effect of temperature and photoperiod on beets will be considered further in this paper.

Induction of flowering under the influence of prolonged low temperature and long photoperiods may be tentatively regarded as a single process and may therefore be designated by a single term. The term "vernalization" has sometimes been used for this purpose, including induction of flowering as it occurs in nature and under artificial conditions, but this term has been applied mostly to thermal induction (*16*) and generally to hastening of flowering by prolonged low-temperature treatment of germinating seed. Furthermore, "vernalization" has also been used (*7, 25, 27*) to include beet-seed treatments involving germination at higher temperatures, even though such treatments do not induce flowering. The term "vernalization" will be used in this paper to mean prolonged low-temperature treatment of seed during germination to hasten flowering. For the broader meaning, including thermal induction and photoperiodic induction, the new term "photothermal induction"[4] will be used. Photothermal induction occurs in beets (*5, 24*), celery (*26*), some wheats (*17*), and many other species.

Photothermal induction of flowering in beets is influenced by genetic factors. Munerati (*20*) investigated an annual beet and showed that a single genetic factor was associated with a clear-cut annual habit. Working with this annual strain obtained from Munerati, Abegg (*1*) demonstrated linkage between the bolting factor *B* and the factor *R* for red hypocotyl color described by Keller (*13*). These findings were helpful in the present study.

"Bolting" as used in this paper is the appearance of the seedstalk or the appearance and development of the seedstalk whether or not flowering is involved.

SCOPE OF STUDIES AND EXPERIMENTAL METHODS

Extensive field observations have been made on commercial and experimental sugar-beet-seed fields in the relatively mild climates of southern Utah, southern California, southern New Mexico, and southern Arizona, and also in the colder climates of northern Utah and southern Idaho, where there is usually some winter snow cover. These observations were started in 1930. More recently, similar observations have been made in Oregon and northern California. All these studies were on plantings made in late summer or early fall and were conducted by the method of overwintering the plants in the field (*21*).

Field observations regarding the factors involved in induction of flowering have also been made on commercial fields of beets grown for sugar production in California and other Western States.

Intensive bolting studies were conducted in field plots at Riverside, Calif., in the years 1935–36, 1936–37, and 1937–38. Shading experiments to modify the natural environment were included in these studies.

Greenhouse studies were made with variations in both temperature and photoperiod. An idea of the temperatures used is given by

[4] This term was suggested to the writers by Dr. E. J. Kraus, chairman, Department of Botany, University

showing means obtained by averaging the daily minimum and maximum temperatures. (See table 6.) In interpreting these averages it should be borne in mind that daily maximum temperatures were frequently reached as peaks caused by brief periods of sunshine before ventilators could be opened. Minimum temperatures were reached much more gradually. In general, comparisons were made between rather widely separated temperature levels where precise control was not essential.

Photoperiods were controlled by using artificial light to supplement the natural day length. Mazda globes with 150- to 500-watt capacity were placed over the plants at distances to give intensities of 10 to 100 foot-candles. The photoperiod most commonly used was between 17 and 18 hours. An 8-hour day and a 24-hour day were also used in a few experiments.

Storage experiments were conducted in darkness with plants of intermediate size, usually with a root diameter of 1 to 3 inches. Usually the storage temperature was maintained between 33° and 36° F., with a few tests at approximately 40°.

Seed was given vernalization treatment in cold storage. In most cases the seed lots were first disinfected with a 10-percent solution of commercial formalin for 10 minutes, then washed and soaked overnight and incubated at room temperature under high humidity until the radicles began to emerge. Then in moist condition the seed was stored in closed containers at 33° to 36° F. The germinating seed was allowed to remain at this temperature for various lengths of time, but in most experiments this treatment was continued about 100 days. The sprouted seed was then planted in the field or in the greenhouse.

Material of known genetic constitution with regard to bolting tendency was utilized in some tests. An annual beet obtained from Munerati and previously investigated (*1*, *20*) was used extensively. A few observations were also made with the annual from Milpitas, Calif. (*3*), and also the more extreme annual type from the Imperial Valley of California (*3*). Greenhouse observations were made with several wild species.[5] Vegetative clones were used for the most critical work, and these were propagated and studied over a period of years.

PHOTOTHERMAL INDUCTION IN ANNUAL AND BIENNIAL BEETS

All varieties of *Beta vulgaris* investigated are long-day plants. The chief difference between biennial and annual beets in regard to photothermal induction is the longer period of low-temperature exposure required for flowering in biennials. There is also much variability among biennial types with regard to this requirement, and commercial varieties are made up of a mixture of such types. In biennial beets, photothermal induction is frequently reversed by increasing the temperatures (fig. 1) or by decreasing the length of the photoperiods. This reversal causes the plants to turn vegetative after they have started to develop in the direction of flowering. In annual beets, also, photothermal induction is reversible, but with annuals the influence of photoperiod is stronger than the influence of temperature.

To induce bolting in biennials when grown in the greenhouse with 17- to 18-hour photoperiods it was necessary to hold the average

[5] These wild species were obtained from Dr. G. H. Coons, principal pathologist, Division of Sugar Plant

temperature under about 58° F., although temperatures somewhat higher were effective with continuous light (a 24-hour day), as previously reported by Steinberg and Garner (*24*). All varieties, including annuals, remained vegetative in an 8-hour day under relatively warm temperatures.

Other *Beta* species are also long-day plants. *Beta maritima* L., *B. atriplicifolia* Rouy, the wild or escaped beets of Milpitas, Calif., and the wild beet from the Imperial Valley of California (*3*) are all more or less annual types, but they all remained vegetative in the green-

FIGURE 1.—*Photothermal induction reversed by changes in temperature.* Plants of clone 90 and hence of identical genetic constitution. Seedstalks about 1 inch in height were initiated in both plants under a cool temperature and 17- to 18-hour photoperiod: *A*, Taken to a warm temperature after the initiation of seedstalk, but length of day not changed; *B*, received same treatment as *A* but was returned to the cool temperature after it became somewhat vegetative in the warm temperature.

house under 8-hour days and relatively warm temperatures. The wild species *B. procumbens* Chr. Sm., *B. patellaris* Soland, and *B. webbiana* Moq. developed decumbent branches under both short and long photoperiods, but they failed to produce flowers under the 8-hour day.

The Munerati annual has been investigated (*1, 20*) more carefully han any other annual variety, and its behavior has been relatively miform. It is very sensitive to photoperiod, and at Salt Lake City, Jtah, in a warm greenhouse (60° to 85° F.) it will not start a seedtalk under natural day length until nearly June 1. It is easily held egetative in an 8-hour day at any time of the year under a relatively varm temperature. Plants of this variety, even after 30 days' torage at 33° to 36° F., remained completely vegetative under 8-hour

For 3 years it has been noted that in October plantings at Riverside, Calif., the Munerati annual remains vegetative all winter and the roots sometimes reach 3 or 4 inches in diameter. Under these conditions its bolting tendency cannot be easily distinguished from that of some commercial sugar-beet varieties. In fact, a portion of the plants in the U. S. 12 variety (*22*) initiated seedstalks somewhat faster during the month of March at Riverside than the Munerati annual.

The influence of temperature on the flowering of annual beets is less obvious than that of photoperiod but under certain conditions can be clearly demonstrated. This opinion is based upon detailed studies with the Munerati annual. Two plants of clone 62, a selection from the Munerati annual, initiated seedstalks in 22 days in a warm room with a 17- to 18-hour photoperiod (average maximum temperature 78.0° and average minimum 54.6° F.). Under the same conditions two other plants from the same clone, previously held at approximately 40° for 39 days, started seedstalks in 15 days, or 7 days sooner than those without the low-temperature treatment. In another experiment with the Munerati annual, two groups of plants were compared that had been grown from seed for 69 days at different temperatures. One group had been grown at a relatively cool temperature (average maximum 74.4° and average minimum 43.5°) and the second group had been grown at a relatively warm temperature (average maximum 80.2° and average minimum 52.9°). On December 2, 1937, after the 69-day treatments at these contrasting temperatures, both groups of plants were placed in the same greenhouse compartment under a 17- to 18-hour photoperiod and relatively cool temperature (average maximum 60.0° and average minimum 43.4°). The plants from the cool temperature, although much smaller than those from the warm temperature, all bolted in 79 days, whereas the plants from the warm room required 92 days for all individuals to bolt. Both experiments show that while seedstalk development in this annual is influenced chiefly by photoperiod, nevertheless low temperature also exerts an influence in the same direction

A dominant factor *B* is responsible for the annual habit in the Munerati annual (*1*), but the F_1 hybrids with biennial types, heterozygous for *B* and possibly other complementary genes, are slower to bolt than the annual parent. Some of the annual segregates (having the *B* factor) from backcrosses to biennials are very slow to bolt and may easily be confused with biennials under many combinations of temperature and photoperiod. One annual segregate (5357–1), from a backcross to a biennial from a hybrid with the Munerati annual, was propagated vegetatively for 4 years and studied in some detail. It bolted in midsummer temperatures at Salt Lake City, Utah, under the naturally long days occurring at that time. In this way it could be distinguished from biennials, but under relatively cool or intermediate temperatures (40° to 65° F.) several varieties that are considered biennial bolted just as readily. It is evident, then, that the presence of the *B* factor is most easily distinguished by maintaining relatively high temperatures and a long photoperiod.

Some of the wild annuals from Milpitas, Calif., differ from the Munerati annual in that they will bolt in shorter photoperiods. The population of plants from Milpitas is a mixture of genetic types varying in bolting tendency, but most of the plants are annuals. In

spring plantings at Salt Lake City, Utah, they were quite similar to the Munerati annual, but in an October planting at Riverside, Calif., some of the Milpitas plants bolted much faster than the Munerati annual.

On March 12, 1938, at Riverside, Calif., an interesting comparison was available between the Munerati and Milpitas annuals. The planting was made October 6, 1937. In the Munerati annual, seedstalks were just starting to develop on March 12, 1938, and were not over 2 inches in height, whereas the seedstalks of some of the Milpitas plants were over 3 feet in height on the same date and had well-developed flower buds. It is clear that the short photoperiod during the winter at Riverside was responsible for the delayed bolting in the Munerati annual, and the comparison indicates that some of the Milpitas annual plants do not require as long a photoperiod for bolting as does the Munerati annual. Other experiments have shown that the Imperial Valley annual will flower in still shorter photoperiods than the Milpitas annual. At Salt Lake City, Utah, September 9, 1938, seeds of the Munerati annual and Milpitas and Imperial Valley annuals were planted in flats in a warm greenhouse under the natural photoperiod. By November 11 all of the Imperial Valley annual plants had bolted whereas none of the others showed any bolting.

The facts presented show that while bolting in annual beets is induced mainly by long photoperiods, the process is also influenced by low temperature. The evidence also shows distinct differences in bolting tendency between different types of beets. Some are influenced more strongly by photoperiod and others more strongly by low-temperature exposure.

EFFECT OF PROLONGED LOW-TEMPERATURE EXPOSURE

The induction of flowering in beets by prolonged low-temperature exposure has been observed in experimental field plantings at Riverside, Calif., made in September, October, and November. Here the climate is such that the plants grow all winter, and temperatures during December, January, and February are in the main favorable for induction of flowering. The photoperiod during these months is, of course, short. All the varieties of beets, including some annuals, when grown from seed planted in the fall, remained vegetative in these tests until about March 15 or later, and some of the more vegetative varieties never developed more than a trace of bolters. The behavior was different when plants previously stored for a long period at low temperatures were planted. Plants selected June 2, 1937, for nonbolting, from a strain planted as seed on October 13, 1936, were stored at 36° to 38° F. from June 2 to November 4, 1937, and then transplanted in the field. On January 20, 1938, 7 out of 31 of these beets, or 23 percent, had started to bolt and in one case a stalk was 17 inches high. Later in the spring all the plants flowered normally. The same variety planted as seed in the field October 6, 1937, did not bolt until April 1938. Less than half of the plants in this lot bolted, and none flowered completely. Obviously, induction of flowering was strongly influenced in the plants given prolonged low-temperature exposure.

Evidence of a similar nature was observed with the nonbolting variety U. S. 15. The seed was planted in a 5-acre field near River-

side, October 15, 1935, and a part of the field was maintained 2 years. There was less than 1 percent bolting in the spring of 1936, and in 1937 also most of the plants remained vegetative or bolted too late to produce seed. Roots taken from this field were stored at 36° to 38° F. from June 16 to November 1, 1936, and then planted in an experimental plot at Riverside.

On the same date, November 1, stecklings were transplanted directly from the 5-acre field to the experimental plot. By February 5, 1937, seedstalks had started on two of the plants that had been stored at low temperatures. By March 2, several more showed short seedstalks, and on May 11 all 44 of the plants had bolted, and with fair uniformity in that all but one had buds formed or open flowers. Out of 49 plants transplanted in November directly from the field, only 28 produced seedstalks, and these were extremely irregular in development. Here

FIGURE 2.—Short seedstalks produced in an unfavorable environment after a long period of low temperature storage.

again, prolonged low-temperature exposure resulted in earlier and more complete flowering.

Greenhouse studies showed that after prolonged low-temperature treatment stecklings or mother beets may be so strongly inclined toward flowering that they will develop seedstalks a few inches in length in an environment unfavorable to photothermal induction. Beet 138, selected in 1932 and maintained as a clone since that time, was studied in detail in relation to the length of low-temperature treatment required for initiation of the seedstalk. No. 138 is a nonbolting type and remains vegetative under many conditions where most beets develop seedstalks. A 3-month treatment at 33° to 36° F. did not induce bolting in beet 138 except when followed by a favorable bolting environment, a long photoperiod, and cool temperature. In an experiment started June 6, 1935, a supply of thrifty plants grown from cuttings of No. 138 was taken to cold storage and held at 33° to 36°. On November 25, after a treatment for 172 days, these cuttings were removed from cold storage and planted in the greenhouse in two environments. One environment was favorable to bolting, with a relatively cool temperature (40° to 65°) and a photoperiod of 17 to 18 hours, while the other was unfavorable, with a relatively warm tem-

perature (60° to 75°) and with only the natural light of the short winter days.

By January 15, 51 days after planting, seedstalks had appeared on all the plants in both environments. In the favorable environment the plants continued normal reproductive development, whereas in the warm room the seedstalks turned vegetative after making a short growth and before developing any visible signs of flowers. The two most vigorous plants of the seven in the warm room developed seedstalks 7 to 9 inches in height. A comparable experiment with another variety gave similar results, and the short vegetative seedstalks produced in the unfavorable environment are shown in figure 2. It is evident that the prolonged low-temperature treatment not only prepared the plants for bolting but actually induced a reserve of some sort, so that the reproductive tendency continued for a time in spite of an unfavorable environment.

In other experiments prolonged low-temperature treatment was followed by a controlled 8-hour day under warm temperature, making the environment still more unfavorable for bolting. Out of 20 stecklings of U. S. 33 (3407) (*22*) stored 246 days at 33° to 36° F., 3 plants developed 2-inch seedstalks.

EFFECT OF SHADE UNDER FIELD CONDITIONS

Early in the work of breeding sugar beets for curly top resistance, difficulty was encountered in producing seed at Riverside, Calif. Field observations in this mild climate from 1919 to 1928 revealed that better flowering resulted in the colder seasons and that in the warmer seasons, when dormancy in peaches and walnuts was seriously prolonged, flowering in sugar beets was unsatisfactory.

Two simple experiments in regard to the effect of temperature on flowering in sugar beets were conducted at Riverside in the winter of 1928–29. In one case a portion of two short rows planted late in the fall was shaded through the winter months by means of unbleached muslin. The cloth was on a frame that rested on the ground on the south side of the rows and leaned to the north, so that no direct sunlight could reach the beets or the soil in the protected part of the plot. Soil temperatures at a depth of approximately one-half inch in the shaded and in the unshaded soil were recorded by means of thermographs. The records were not taken during the entire period, but the difference between the temperatures in the two environments from January 8 to March 1, a period of 1,248 hours, is indicative of the contrast in conditions to which the shaded plants and those in full sunlight were exposed. During the period mentioned the soil temperature in the shade was continuously below 60° F. During this same period the temperature of the unshaded soil was above 60° for a total of 289 hours, or 23 percent of the total time. Bolting counts on June 4, 1929, revealed that in the unshaded plants 62 of the 347, or 18 percent, had bolted, while in the shaded plants 50 of the 130, or 38 percent, had bolted.

Another test in the same winter involved growing one lot of potted plants in full sunlight and another lot on the north side of a nursery lath house, where the plants were protected from direct sunlight throughout the winter months. Owing to the elevation of the site and consequent air drainage, the temperatures were relatively warm

Some of the pots in each case were submerged in the soil and others were left on top of the ground. There was only a trace of seedstalk development in the plants exposed to sunlight, while nearly all the shaded plants developed vigorous seedstalks. Soil temperature records were not taken. The temperature differences probably corresponded to those in the previous experiment.

The results of these two tests indicate that in mild climates, such as that of Riverside, Calif., induction of flowering was increased when the beets were kept cooler during the winter by means of shade.

As a result of renewed interest in the factors involved in induction of flowering in beets, a field experiment with artificial shade was started at Riverside, Calif., in the fall of 1936, to get further evidence

FIGURE 3.—*Shade in relation to soil temperature and bolting. Horizontal shading frames in foreground; vertical shading frames in background. (Photographed November 26, 1937.)*

on the influence of temperature. The beets were planted September 18 on single-row beds running east and west. Portions of these rows were then shaded by frames made of shingles coated on both sides with aluminum paint. The frames were placed in series, so that they would shade the beet rows to the north and reflect light on the rows to the south. This vertical type of frame, together with a flat or horizontal type used in a later experiment, is shown in figure 3. There were three different treatments with the vertical frames, as follows: (1) Portions of rows were shaded only during the warmer part of the fall, September 18 to November 12, 1936; (2) other portions of rows were shaded only during the colder part of the season, November 12, 1936, to April 1, 1937; and (3) other portions of rows were shaded from September 18, 1936, to April 1, 1937. Five varieties, all rather low in bolting tendency, were used. There were duplicate plots for each shading treatment. Soil temperatures close to the

taken in the shaded and unshaded plots by means of soil thermographs Temperatures of the beet crowns at about one-half inch below the soil surface were determined by means of chemical thermometers inserted snugly into holes made with a cork borer in the sides of the roots. The temperatures of the roots were almost the same as the soil temperature.

Bolting counts on May 10, 1937, gave the following averages: Unshaded, 66 percent; shaded (1) September 18 to November 12, 65 percent, (2) November 12 to April 1, 89 percent, and (3) September 18 to April 1, 79 percent. Temperature records are given in table 1. Shading during the warmer part of the fall did not increase bolting and may have been detrimental in some way. Shading during the colder weather increased bolting.

TABLE 1.—*Temperatures of shaded and unshaded soil at one-half-inch depth and percentage of bolters in varieties 550 and 617 with and without artificial shade*

Treatment	Temperature: Daily average [1]	Temperature: Mean daily deviation: Above average	Temperature: Mean daily deviation: Below average	Plants bolting [2]
	°*F.*	*Day degrees* [3]	*Day degrees* [3]	*Percent*
Nov. 23, 1936, to Mar. 21, 1937:				
No shade	49.16	5.13	5.22	8[illegible]
Vertical shade	43.59	3.41	3.60	100
Nov. 22, 1937, to Mar. 20, 1938:				
No shade	51.34	3.09	3.02	47
Vertical shade	50.51	2.40	2.61	7[illegible]
Horizontal shade	52.17	1.90	2.01	81

[1] Average temperature determined by planimeter measurements of thermograph charts.
[2] Averages of the two varieties.
[3] "Day degree" is defined as a measure of accumulated temperature and represents an average of 1 degree of deviation from the base temperature during a period of 24 hours. Day degrees were measured as areas above and below the base temperature on thermograph charts by means of a planimeter.

Another shading experiment in the field was started at Riverside in the fall of 1937. Vertical frames, of the type used in the previous experiment, and horizontal frames were used. The latter were made of heavy kraft paper coated on both sides with aluminum paint and supported on frames of wood and wire. These frames were 18 inches wide and 12 feet long. The beet rows were 20 inches apart, so that when the frames were laid between the rows nearly all the soil between the rows was shaded (fig. 3). The beets were planted October 6, and the shading was started November 19. Two of the varieties included in the previous year's test, 550 and 617 (U. S. 14), were used in the experiment. These two varieties have practically the same bolting tendency. The shading was discontinued March 21. The results in percentage of bolters and the soil-temperature records are summarized in table 1. The bolting percentages are averages for the two varieties used.

The percentage of bolting was higher in the shaded than in the unshaded plots each season (table 1), and higher in the colder season of 1936–37 than in 1937–38 (table 1).

The fact that in the second season more bolting occurred in the plots with horizontal shades than in the unshaded plots was probably due to the temperature having been lower in the fall and early winter in the shaded plots (fig. 4). The mean temperature for the 7 weeks

unshaded plots than in the plots with horizontal shades. The accumulated deviation above 51.19° (the mean for the plots with horizontal shades) was 149.49 day degrees greater in the unshaded plots; the accumulated deviation below 51.19° was practically the same for the shaded and unshaded plots. It is thus evident that during the late fall and early winter the beets under the horizontal shades were in a colder soil environment than were the unshaded beets. Later, as the leaves grew larger and shaded the soil more effectively, the mean soil temperature in the unshaded plots dropped a little lower than in the plots with horizontal shades. However, the soil temperatures in both plots were favorable to thermal induction during most of the latter part of the winter. The fact that the horizontal shades kept the soil temperatures relatively low in late fall and early winter apparently allowed

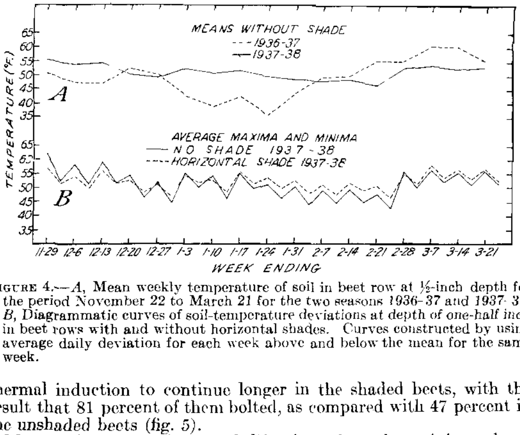
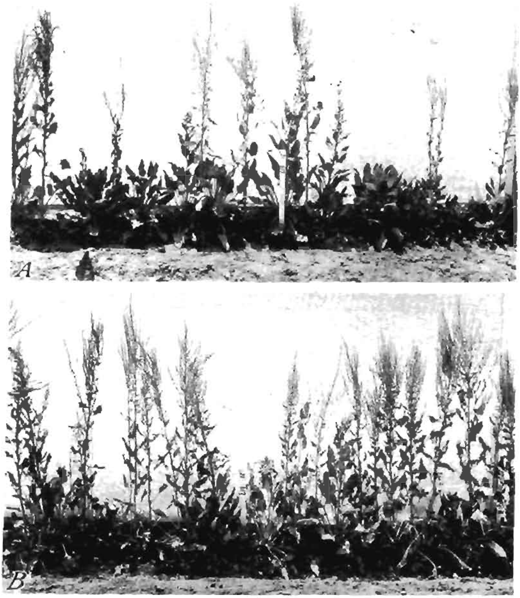


FIGURE 4.—*A*, Mean weekly temperature of soil in beet row at ½-inch depth for the period November 22 to March 21 for the two seasons 1936–37 and 1937–38. *B*, Diagrammatic curves of soil-temperature deviations at depth of one-half inch in beet rows with and without horizontal shades. Curves constructed by using average daily deviation for each week above and below the mean for the same week.

thermal induction to continue longer in the shaded beets, with the result that 81 percent of them bolted, as compared with 47 percent in the unshaded beets (fig. 5).

Many field observations in California and southern Arizona have revealed that with rows running east and west the north sides of two-row beds have more bolters than the south sides. For example, in a field of the low-bolting variety R. & G. Old Type, planted near Calexico, Calif., October 8, 1937, there was a striking difference between bolting on the north and south sides of the beds. On May 15, 1938, there were 37 percent bolters on the north side of the beds and 7 percent on the south side. The temperature of the soil and consequently that of the beet crowns at the same level was often 4° to 5° F. cooler on the north side of the beds. In the field at Calexico the leaf area on the south side of the beds was larger, which gave that side an advantage in regard to induction by light, if it is assumed that leaf area is a factor in this process in beets. It seems probable, therefore, that the greater amount of the bolting on the north side of the beds resulted from induction by temperature.

These observations and experiments gave evidence of the relation of the temperature of beets, as influenced by the temperature of the surrounding soil, to bolting. In mild climates where winter temperatures may be too high for flowering or even for bolting, more bolting and flowering result from a slight lowering of the temperature for a pro-



'IGURE 5.—Bolting in shading experiment at Riverside, Calif., with variety U. S. 14, planted October 6, 1937. Photographed June 7, 1938. *A*, Without artificial shade; *B*, soil between rows shaded with horizontal shades of aluminum-painted paper from November 22, 1937, to March 20, 1938.

onged period. Shading the soil surrounding the beets during the vinter keeps the beets enough colder to increase bolting.

VERNALIZATION OF SEED AND EFFECTS OF IRREGULAR GERMINATION

Increased bolting in sugar beets as a result of subjecting germinating eed to low-temperature treatment has been previously described

(*7, 12, 25, 27*). Numerous tests by the writers showed increased bolting in plants grown from seed that had been subjected to vernalization treatment, but in some instances no effect was observed. Results of the following experiments explain this apparent inconsistency.

In May plantings at Salt Lake City, Utah, of seed subjected previously to vernalization treatment, little or no increase in bolting occurred. A considerable increase in bolting was observed, however, when a comparison of vernalized and untreated seed was made in an early March planting (table 2). This difference is to be explained by the fact that there was additional thermal induction in the field in the March plantings and this influence added to that accomplished by vernalizing the seed resulted in the effect noted. Seed that had been subjected to vernalization treatment was planted at Riverside, Calif., in October. There was no increase in bolting the following spring. Evidently the relatively warm weather in the fall resulted in enough reversal of the inductive process to overcome the effect of the seed treatment.

TABLE 2.—*Increased bolting from vernalized seed in a field planting made Mar. 16, 1937, at Salt Lake City, Utah*

Variety	Seed treatment	Plants observed	Plants bolting
		Number	*Percent*
U. S. 12 (618)	No treatment	95	1.05
Do	Washed, incubated and dried, but not vernalized	96	5.20
Do	Washed and incubated and then vernalized at 33° to 36° F. for 61 days.	162	12.95
U. S. 14 (617)	do	122	22.20

Striking results with seed vernalization were obtained in the greenhouse under relatively cool temperatures and with a 17- to 18-hour photoperiod when seed subjected to vernalization treatment was planted in comparison with untreated seed. Two strains low in bolting tendency, 2168 and 4919, and the easy-bolting biennials U. S. 12 and 3401 were used. In addition to these varieties, nonvernalized seed of U. S. 1 and the Munerati annual were included in the test. The results are given in detail in table 3. Eighty-two days after planting, untreated seed of U. S. 12 had given rise to 2.8 percent bolters and the vernalized seed had produced 14.3 percent bolters. With 3401, the untreated seed had given rise to no bolters, while the vernalized seed had produced 36.1 percent bolters. At this time there were no bolters in 2168 and 4919, even with the vernalized seed. Vernalization increased the rate of bolting in the easy-bolting biennials, but did not sufficiently affect the two varieties inherently low in bolting tendency to cause them to bolt in the 82-day period.

The relationship of sprouting to vernalization has been noted in several instances. In a series of experiments in 1933 and 1934, where dry seed was planted in moist soil in flats and held at approximately 40° to 45° F., the germination of most varieties was noticeably irregular. After all sprouted seed in some of the flats was killed by drying and freezing, these flats were watered and placed under temperatures favorable for germination and growth of the surviving seed. The seed that had not sprouted during the vernalization treat-

ment was then found to be unaffected. Similar seed that sprouted during the vernalization treatment was thermally induced to a considerable extent.

TABLE 3.—*Greenhouse test with vernalized seed* [1]

Variety or strain	Plants bolting from—	
	Nonvernalized seed	Vernalized seed
	Percent	*Percent*
2168	0	0
4919	0	0
U. S. 12 (4900)	2.8	14.
3401	0	36.
U. S. 1 (9153)	7.5	----------
Munerati annual (2240)	100.0	----------

[1] Vernalized seed was incubated a short time at 70° F. and then held at 33° to 36° for 55 days previous to planting. There were 8 replications of each variety and treatment. Each replication consisted of a single row 30 inches long with 20 to 30 plants to the row. Planted Sept. 24, 1934; bolters counted Dec. 15, 1934.

In a field planting at Salt Lake City made March 16, 1937, vernalized seed of U. S. 12 was compared with two kinds of nonvernalized seed (table 2). Lot 1 of the nonvernalized seed was wholly untreated; lot 2 was washed, incubated at 70° F., and finally dried. This treatment of lot 2 hastened germination in the field during the relatively cold weather of early spring and thereby resulted in more thermal induction, and consequently the bolting was increased. However, the vernalized seed gave more than twice as many bolters as did lot 2 of the nonvernalized seed.

In this same field planting, a vernalized lot of seed of the nonbolting variety U. S. 14 gave even more bolters than the vernalized seed lot of U. S. 12 (table 2). It is well known from experience that U. S. 12 is much more likely to bolt in the field than U. S. 14 (*22*). Before planting it was observed that the vernalized seed of U. S. 14 had germinated to a greater extent than the vernalized seed of U. S. 12. This greater germination during the vernalization treatment probably accounts for the fact that plants grown from the vernalized seed of U. S. 14 bolted more than those grown from the vernalized seed of U. S. 12.

In preparing the seed for vernalization treatment, it was first thoroughly soaked and washed and then held at about 70° F. until the majority of the seed balls showed radicles about 1 mm. in length. It was difficult, however, to obtain a uniform degree of germination, and this lack of uniformity in germination was still more noticeable after a 60-day treatment at 33° to 36°. Some radicles were over an inch in length, while other seeds showed no evidence of germination.

The results of an experiment to discover the extent to which sprouting influences thermal induction in seed are given in table 4. Seed previously moistened and then held at 33° to 36° F. for 55 days was sorted into three classes. Class 1 included seed balls with no visible radicles; class 2 those with such radicles as were evidently less than 3 mm. in length; and class 3 those with all radicles developed and all over 3 mm. in length. Bolting counts 82 days after planting revealed approximately twice as many bolters in classes 2 and 3 as in class 1.

The increased bolting from the seed with visible radicles is highly significant (table 4).

Heritable differences complicate the problem of thermal induction in seed. Strain 2168, for example, was found to sprout more rapidly at low temperatures than strain 3401. It might be expected, therefore, that when treated similarly strain 2168 would be more effectively vernalized. Contrary to such expectation, vernalization may not cause any bolting in strain 2168, but it may cause abundant bolting in strain 3401 (table 3). Such results are explained by inherent differences in bolting tendency. Strain 2168 is inherently a nonbolter, while strain 3401 is an easy-bolting strain. If one of two strains of the same bolting tendency germinated more readily than the other at low temperatures, it would be expected to respond more strongly to vernalization.

Vernalization increased the rate and percentage of bolting provided that (1) the seed sprouted during the cold treatment and (2) the vernalized seeds were planted in an environment favorable enough for induction of flowering to continue. Vernalization did not overcome inherent differences in bolting tendency.

TABLE 4.—*Effect of extent of sprouting on the vernalization of seed* [1]

Strain	Plants bolting from seed showing indicated extent of sprouting		
	No radicles visible	One or more radicles visible on each seed ball but less than 3 mm. in length	All radicles visible and over 3 mm. in length
	Percent [2]	*Percent* [2]	*Percent* [2]
3401	25.9	52.2	60.9
3410 (parent of U. S. 12)	28.8	54.3	53.5

[1] Seed all vernalized in the same container, but seed balls separated and classified at the end of the treatment according to the extent of sprouting. Seed planted in rows 30 inches long with about 20 plants per row. Rows spaced 4 inches apart with 5 replications of each seed classification for each variety. Planted Sept. 24, 1934, and bolters counted Dec. 15, 1934.

[2] Standard error of difference due to extent of sprouting when planted=7.9 percent.

A NEW GENETIC FACTOR FOR BOLTING

The action of certain genes in relation to the physiological process responsible for flowering should be known for a better understanding of photothermal induction. The factor B' now to be described has an important role in the process in beets. It is responsible for an easy-bolting tendency but appears to differ from the factor B described by Munerati (*20*) and Abegg (*1*), because plants carrying B' remained vegetative under field conditions, whereas plants carrying B were strictly annual. It will be seen that B' and B are regarded as allelic factors and dominant to the factor b associated with a nonbolting tendency. Linkage discovered between B' and the color factor R (*13*) made the genetic analysis possible. In order to study the factor B', it was necessary to give careful attention to environmental conditions affecting photothermal induction as well as to the genetic material to be investigated.

PARENTAL MATERIAL

The parental material consisted of three clones selected in 1932 and subsequently studied in detail. Their bolting tendencies were therefore well known. Clone 70, *rrB′b*, provided the factor *B′* for bolting, while clone 79, *RRbb*, and clone 90, *rrbb*, were vegetative types. These clones were selected from rather closely bred lines that had been characterized by a fair degree of uniformity.

Clone 70 was selected from2769, a strain obtained from William W. Tracy, Jr. This plant was a fast-bolting biennial. Experience with vegetative cuttings from it, and with seed from its parental strain and its selfed progeny, showed that it was not an annual under field conditions at Salt Lake City, but it was a fast bolter and was easily confused with annuals under relatively low temperatures in the greenhouse. Clone 70 also carried a genetic factor for self-fertility, which distinguished it from most sugar beets.

Clone 79, *RRbb*, was a strongly vegetative or nonbolting type. It was first propagated vegetatively because of interest in its male-sterile character, which is a convenience in hybridization work. The plant was apparently normal in every other respect, but the pollen grains were completely aborted.

Clone 90, *rrbb*, came from Salt Lake City strain 2167, a curly top-resistant strain with a strongly vegetative or nonbolting tendency. An advantage of clone 90 for crossing is its strong self-sterility, for when it is used as a female parent one can be sure of obtaining hybrids without taking the trouble to emasculate.

METHODS

The male-sterile clone 79, *RRbb*, was pollinated from clone 70, *rrB′b*, and several F_1 plants were grown. The F_1 plants segregated for male sterility and self-fertility. F_2 progenies were produced from some of the self-fertile F_1 plants. Backcross progenies were also produced by hybridizing clone 90 (*rrbb*) with pollen from a few of these F_1 plants selected at random from those producing normal pollen.

Respective progenies were tested for bolting by subjecting some of the seed to a vernalization treatment and growing the plants in the greenhouse. The seed was vernalized for 101 days at 33° to 36° F. At planting time most of the seed had sprouted and the radicles were ıpproximately 0.5 to 2 cm. in length. Some seed lots were more ıniform in sprouting than others, and some were affected by the levelopment of mold. Seed of the backcross progeny (4615), vhich gave the most interesting results (table 5), however, was nearly ree from mold, and the radicles were more uniform in length than in nost of the other progenies. This proved to be a fortunate circumtance, because the uniformity of the vernalized seed when planted is elieved to have had an important bearing upon the clear-cut diferentiation secured for the bolting types in this progeny (table 5).

The plantings were made in greenhouse beds 30 inches wide with ows 4 inches apart. Progeny 4615 was planted in 15 of these rows, nd 250 plants, or about 17 plants per row, grew from uniformly paced seed. Owing to limited space, plants of other progenies were ore closely spaced, with about 34 plants per row. Nonvernalized eed of the respective progenies was planted at the same time for comarison. Artificial light was used to supplement the regular day

length to make approximately a 17-hour day. The temperature controlled rather roughly, and a record was taken each day fro minimum and maximum thermometer.

TABLE 5.—*Correlation between time of bolting and hypocotyl color from back progenies 4615 and 4612*

SEED OF PROGENY 4615 VERNALIZED

Days after planting	Bolters		Vegetative plants		Total plants	Cross-over:	
	Red hypocotyl	White hypocotyl	Red hypocotyl	White hypocotyl			
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Per*
47	29	104	98	19	250	48	19.2
105	29	108	98	15	250	44	17.6
136	71	108	50	14	243	85	35.0
SEED OF PROGENY 4615 NOT VERNALIZED							
136	5	34	75	48	162	53	32.7
217	39	65	41	17	162	56	34.6
235	57	74	19	8	158	65	41.1
SEED OF PROGENY 4612 VERNALIZED							
164	0	2	87	88	177	---	---
217	17	15	70	75	177	92	
257	73	85	14	5	177	78	

[1] Standard errors of cross-over values from Immer's tables (*11*). Immer's probable errors divid 0.6745 give the standard errors.

The growing plants were carefully watched, and where possible temperatures were increased or decreased to produce what was belie to be the optimum temperature to differentiate plants with bol tendencies from strongly vegetative or nonbolting types. It wil noted in table 6 that temperatures increased slightly in October again in January, decreased in March and April, and increased ag in May. The distinct decrease in temperatures for March and A was the result of manipulation required to bring out more bolt after a fairly good separation between bolting and vegetative ty had been made in the winter months.

TABLE 6.—*Daily greenhouse temperatures, 1934–35*

Month	Temperature		
	Average maximum	Average minimum	Me
	° *F.*	° *F.*	°
September 24 to 30	75.1	40.7	
October	81.3	47.0	
November	73.9	47.0	
December	71.8	53.0	
January	75.3	55.5	
February	71.0	53.7	
March	62.4	42.0	
April	72.1	43.0	
May	80.5	46.1	

Bolting records were taken at intervals as bolters appeared, and in most cases the bolters were pulled when counted in order to allow more space for the remaining plants.

EXPERIMENTAL RESULTS

The extreme differences in bolting due to heredity are shown in figure 6. The Munerati annual plants had all started seedstalks within 50 days after planting, whereas the vegetative strain 2167 showed only a small percentage of bolting 200 days after planting. The selfed progeny of clone 70 was between these two extremes but somewhat closer to the early-bolting side. This performance was in agreement with considerable additional evidence that clone 70 represented a rather fast-bolting type.

Although the plants in the selfed progeny of clone 70 were fast bolters there was considerable variability, as shown in figure 6. Some

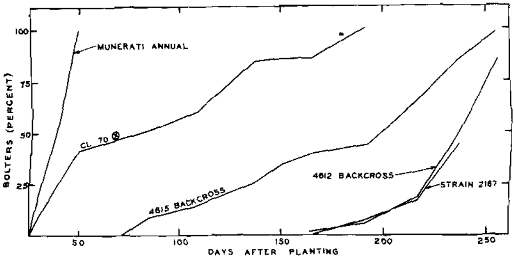


FIGURE 6.—The gradation of beet types from strong annual to strongly vegetative biennial habit is shown in this graph summarizing results obtained with nonvernalized seeds. Clone 70 ⊗ indicates selfed progeny of clone 70. Clone 70 ⊗ and strain 2167 represent parental types, and 4612 and 4615 are backcross progenies from hybrids between clone 70 and clones from strain 2167 described in test.

plants in the progeny bolted much faster than others, but it is uncertain how much of this was due to heredity and how much to environmental differences between different plants. The question of whether or not clone 70 was homozygous for bolting tendency is made clearer by studying its hybrid progenies. Clone 70 is represented by backcross progenies numbered 4612 and 4615 and F_2 progenies numbered 4608 and 4609. The heterozygosity of clone 70 is believed to be shown in the performance of these backcross and F_2 progenies. For the backcross progenies, figure 7 and table 5 illustrate the fact that 4615 segregated for fast-bolting types while 4612 was almost equal to the vegetative parental types. Likewise, the data in table 7 show that the F_2 4608 was fast in bolting while the F_2 4609 was much slower. It seems probable that clone 70 was of heterozygous constitution, $B'b$, assuming B' to be responsible for the bolting tendency. When crossed to clone 79 (*RRbb*) the F_1 plants would be expected to be $RrB'b$ and *Rrbb* in equal proportion. Backcrossing the F_1 plants at random to clone 90 (*rrbb*) would then yield some backcross progenies

like 4615, consisting of both *B'b* and *bb* plants that show sharp segregation for bolting and vegetative types, and other backcross progenies like 4612, all *bb*, which lean rather strongly to the vegetative side. Likewise in F_2 progenies, data presented in table 7 indicate that progeny 4608 was derived from a plant of constitution *RrB'b* and progeny 4609 from a plant of constitution *Rrbb*.

LINKAGE BETWEEN *B'* AND *R*

The correlation between bolting tendency and hypocotyl color is a point of much interest. This correlation is shown for the backcross progeny 4615, clone 90, *rrbb*, ×(clone 79×clone 70), *RrB'b*, in figure 7 and table 5. Figure 7 shows, especially with the vernalized seed, than the plants with white hypocotyls (*rrB'b*) bolted much more rapidly that those with red hypocotyls. Evidently because of their genetic constitution, the white hypocotyl plants were in large part so strongly affected by the seed treatment that they bolted quickly after planting. Since the bolting tendency was associated with the

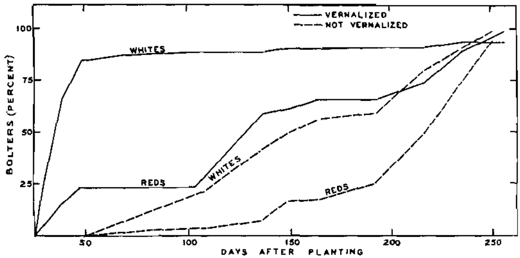


FIGURE 7.—Bolting reaction of color types in backcross progeny 4615.

recessive hypocotyl color in the clone 70 parent, it can be assumed that the respective genetic factors responsible for these characters were linked in their inheritance.

A cross-over value of 15.5 percent between the factor *R* for hypocotyl color and the factor *B* for annual habit has been reported by Abegg (*1*). In table 5, progeny 4615 shows a cross-over value of 17.6 percent between *R* and *B'* at 105 days after planting, which is very close to Abegg's value. Since the rate of bolting is perhaps not entirely clear-cut, even at 105 days after planting, it may be assumed that a more perfect separation between bolting and vegetative types would lead to a slightly lower figure than 17.6 percent crossing over. Calculations from figures obtained from a less desirable classification, made at 136 days after planting, give 35 percent crossing over, which is a poor estimate because of an excess of observed plants in the bolting class over the expected proportion. It is assumed from the similar association of *B'* with *R*, and of *B* with *R*, that *B* and *B'* may be allels with identical positions on the chromosome. There is, however, no critical proof that *B* of the Munerati annual and *B'*

of clone 70 are not the same gene with the apparent difference between them caused by additional modifying factors.

The F_2 progenies were not expected to yield data that would be as clear-cut as those from the backcross progenies. This was particularly true because in the F_1 cross, clone 79 $(RRbb) \times$ clone 70 $(rrB'b)$, the characters entered in the repulsion phase. However, table 7 shows that a significant negative correlation was obtained between bolting and hypocotyl color in F_2 progeny 4608. The χ^2 value has been used to measure this correlation in table 7. This value is highly significant, but it does not give an accurate idea of the percentage of recombinations.

TABLE 7.—*Correlation between time of bolting and hypocotyl color from F_2 progenies 4608 and 4609* [a]

PROGENY 4608

Days after planting	Bolters		Vegetative plants		Total plants	χ^2 [b]
	Red hypocotyl	White hypocotyl	Red hypocotyl	White hypocotyl		
	Number	*Number*	*Number*	*Number*	*Number*	
40	77	36	387	89	589	9.461
85	171	75	282	40	568	28.186
172	235	85	162	26	508	11.244

PROGENY 4609

Days after planting	Bolters: Red hypocotyl	Bolters: White hypocotyl	Vegetative plants: Red hypocotyl	Vegetative plants: White hypocotyl	Total plants	χ^2 [b]
39	6	4	282	90	382	-
108	69	25	219	69	382	0.266
192	161	53	127	41	382	.007
257	276	90	12	4	382	-

[a] All data are from vernalized seed of progenies 4608 and 4609.
[b] According to Fisher's tables (*8*), with 1 degree of freedom χ^2 should exceed 6.635 for the 1-percent point of significance.

DISCUSSION AND CONCLUSIONS

Initiation of seedstalks and flowering of biennial beets is brought about mainly by the cumulative effect of low-temperature exposure followed or accompanied by the effect of long photoperiods. The effect of temperature may be as pronounced as the effect of photoperiod. With beets it is difficult in many cases to distinguish between these two principal environmental effects; hence "photothermal induction" is used in this paper to denote induction of flowering and includes both photoperiodic and thermal induction.

Thermal induction and photoperiodic induction probably result in the production of one or more flower-inducing substances, or hormones, as indicated by work with other plants by Čajlachjan (*2*), Moškov (*19*), Melchers (*18*), Loehwing (*15*), and by one of the writers with beets.[6] Prolonged thermal induction in some cases influences the reproductive tendency so strongly that this tendency continues for some time after the environment has become distinctly unfavorable for reproduction. It appears, therefore, that if induction of flowering is caused by some substance, enough of this substance accumulates while temperatures are favorable to last for some time after conditions become unfavorable.

[6] STOUT, M. Unpublished work.

In the reproductive process the role of low-temperature exposure comes in mainly in the initial stages. In biennial beets somewhat higher temperatures are more favorable for growth of the seedstalks and flowering, as has been reported in the case of tulips (*10*) and spinach (*14*). However, continuation of relatively cool temperature is necessary for completion of flowering in beets. Steinberg and Garner (*24*) reported that in a biennial variety of beets seedstalks were initiated and some flowers produced at 73° F. with continuous light, but the photographs published by these writers show that the seedstalks were somewhat vegetative under this relatively warm temperature. Obviously, the induction was inadequate for complete reproductive development. The writers have observed that vegetative seedstalks similar to those pictured by Steinberg and Garner commonly result from inadequate low-temperature exposure during seedstalk initiation or from excessively high temperatures during seedstalk development.

The relative importance of thermal induction and photoperiodic induction varies with different types of beets according to their genetic constitution. A comparison of the biennial U. S. 12 and the Munerati annual in fall plantings at Riverside, Calif., illustrates this point. In early spring, when the days were relatively short, some individual plants of the biennial U. S. 12 showed seedstalks before any stalks appeared in the Munerati annual. Evidently the U. S. 12 plants were so strongly influenced by thermal induction that they started to develop seedstalks during relatively short days that were unfavorable. The Munerati annual, on the other hand, needs little or no thermal induction, but it requires a relatively long photoperiod. Evidently the Munerati annual requires more photoperiodic induction for initiation of flowering than does U. S. 12 or else does not respond to low-temperature exposnre so readily.

The bolting factor *B* (*1, 20*) is responsible for much of the annual tendency in the Munerati annual. All plants with this factor that have been studied critically at Salt Lake City, Utah, have initiated seedstalks and flowered under long days during midsummer temperatures. A factor *B'* with an effect comparable to that of *B* is described in this paper. Plants with this factor, when grown under long days and at warm temperatures, will flower as readily as the Munerati annual if they have had a short treatment with low temperature.

The influence of the genetic factors *B*, *B'*, and *b*, which determine the tendency for bolting in beets, is comparable in importance to the influence of temperature and photoperiod. Furthermore, the discovery of these genetic factors affords a new approach to the study of induction of flowering. It is now clear that this physiological process would be better understood if more knowledge were available regarding the physiological action of the genes. In the case of *Hyoscyamus niger* L., Melchers (*18*) gives evidence for a gene for biennial tendency with physiological significance somewhat similar to that of *B* or *B'* in beets. Genes in soybeans that influence time of flowering also have been reported (*28*), and this fact should be useful in studying the effect of photoperiod peculiar to this plant.

Reversal or prevention of the induction of flowering is an important factor in vegetative development in beets. This reversal or prevention permits continuation of growth and the accumulation of food reserves.

These food reserves are necessary for beets to survive as biennials, and it is because of the abundant supply of stored food in the form of sucrose that sugar beets are of commercial importance as a source of sugar. Reversal of induction of flowering makes possible the perennial tendency in biennial beets that is evidenced in seed fields where many plants survive and resume vegetative development after the seed is harvested. In contrast to the behavior of biennial beets in this respect, the Munerati annual, after seed has been harvested from it in July at Salt Lake City, either dies or produces more flowering branches. Evidently induction of flowering in this annual is stronger than the reverse process, even in midsummer. The reverse process becomes evident in beet-seed fields of the Southwestern States when, owing to the fact that the temperatures have been too warm for adequate induction, some plants with seedstalks several feet in height turn vegetative. The same type of reaction is often evident with bolters in fields grown for sugar. It is on this account that the bolters classed as late are much less objectionable than those described as early (*6*). The flowering process in such plants is reversed, and nearly normal vegetative development of the roots takes place.

A knowledge of the principles of photothermal induction is useful to the plant breeder in breeding for a desired bolting tendency. Irregular germination under temperatures conducive to vernalization causes variability in degree of induction obtained. This may constitute a stumbling block to the breeder who selects for nonbolting without knowing that some of his nonbolting phenotypes failed to bolt merely because they were delayed slightly during the early period of germination. Nonbolting selections from early spring plantings may, on this account, be untrustworthy. Breeding for nonbolting is also handicapped by difficulties in obtaining photothermal induction adequate for propagation of the most extreme nonbolting selections. The strongest nonbolters are usually the poorest seed producers and tend to be neglected or dropped in the breeding process. Optimum and uniform conditions for induction of flowering are therefore required for the best results in developing nonbolting types.

A knowledge of the factors that influence photothermal induction is also of practical importance in the selection and maintenance of beet-seed-growing areas, particularly where the method (*21*) of overwintering the plants in the field is to be used. Success or failure in producing seed of some particular variety may depend on the choice of a suitable area. In some of the areas now growing seed, where temperatures are too high for the best thermal induction, it is important to develop promptly in the fall and hold an extensive growth of leaves to shade the soil and thereby reduce soil temperatures. The relation of the leaf area to induction by light may also be important, but this phase of the problem has not yet been adequately investigated.

SUMMARY

"Photothermal induction" of flowering is a new term used to signify induction of flowering by both light and temperature. In beets the effect of photoperiod was found to be intimately associated with and dependent upon temperature exposure. The effect of low-temperature exposure favorable to subsequent flowering was demonstrated

with germinating seed, with beets kept for a time in cold storage, and with growing plants.

Some of the factors that influenced bolting in the field acted indirectly by altering the range or duration of effective temperatures. Thus, shade increased bolting by lowering the temperature of the soil and consequently the temperature of the beet crown under conditions where the unshaded soil was too warm. Irregularity in germination of seeds under temperatures conducive to induction of flowering resulted in variation in bolting because of the fact that seeds retarded in sprouting escaped some of the low-temperature influence.

Genetic variability in beets with regard to response to temperature and photoperiod was shown in both annuals and biennials. A factor for bolting was identified, which is designated B' and is regarded as allelic to the factor B discovered by Munerati (*20*) and further described by Abegg (*1*). Identification of the factor B' was accomplished by hybridizing selected parental material and testing the backcross progenies under controlled environmental conditions.

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EFFECT OF BLUE STAIN ON SPECIFIC GRAVITY AND STRENGTH OF SOUTHERN PINE[1]

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INTRODUCTION

Blue stain, because of its widespread and common occurrence in the sapwood of logs and lumber, has been the subject of numerous investigations to determine its effect on the strength of wood. Among the earlier studies, between the years 1897 and 1911, were those by Rudeloff (*10*),[3] Von Schrenk (*12*), Münch (*9*), and Weiss and Barnum (*14*). The entire group included tests of compression parallel to the grain, bending strength, toughness, and hardness. The results were variable and on the whole rather inconclusive, probably owing in considerable measure to differences in the original mechanical properties of the wood and in the moisture content of the specimens at the time of testing. None of the results obtained in the several tests suggested that any practically significant weakening resulted from blue stain.

During the present decade the effect of blue stain on the strength of wood has received further attention, in most cases with more precise procedures to reduce the influence of factors other than the blue stain. In tests by Mayer-Wegelin and coworkers (*8*), Vanin (*13*), Chapman (*2*), Findlay and Pettifor (*3, 4*), and Jalava (*7*), due consideration was given to the moisture content of the wood and, with the exception of that by Mayer-Wegelin et al., all or a substantial part of the work was done on matched [4] specimens in which stain had been produced by artificial inoculation and laboratory-controlled incubation. The results of these tests corroborated in general the findings of the earlier ones but disclosed definitely that blue stain may reduce the toughness of wood considerably. Except in one study by Findlay and Pettifor (*4*), on a tropical wood (obeche), compression and bending strength, hardness, and specific gravity have been reported as but slightly or not measurably affected.

The tests reported in this paper were conducted by the senior author and analyzed by the junior author. They conclude a series, the first results of which were published in 1933 (*2*). The aim of the additional testing was to gather evidence on additional fungi.

MATERIAL AND METHODS

SELECTION AND CUTTING OUT OF SPECIMENS

The test specimens were obtained from green logs of southern pine, taken at a height of 8 to 16 feet in the trees. The wood chosen was

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[3] Italic numbers in parentheses refer to Literature Cited, p. 132.

[4] The specimens were matched individually or by groups, by sawing them out of the same piece of wood in such a manner as would minimize the influence of the initial variation in physical properties.

straight-grained, clear throughout, and of medium density and uniform growth. The specimens were sawed to a size of ¾ by ¾ by 10 inches in such a manner as to present as nearly as possible true tangential and radial faces. Each specimen to be inoculated with a staining fungus was directly matched with a control specimen, either tangentially or longitudinally. The pattern of matching varied somewhat among the several tests, depending chiefly on the number of strength properties investigated at one time. However, there was no important deviation from the basic matching schemes, the essential features of which are illustrated in previous papers by the authors (*2*, *11*).

HANDLING AND STAINING OF SPECIMENS

All but certain specimens, which will be noted later, were maintained in the green condition, usually well above the fiber-saturation point, until the tests were concluded. Some of the tests were made on sticks that had been given no sterilization treatment prior to inoculation but, in order to insure pure cultures, most of the sticks were given a steam-heat treatment of 30 minutes at 100° C. and a few were heated for 5 minutes at a steam pressure of 12 pounds. Both the sticks that were subsequently stained and the controls were given the same heat treatment. In the absence of heat treatment, aseptic preparatory procedures and extra heavy inoculation were depended upon to reduce contamination. Visible contaminations were comparatively few and when they did occur the affected sticks were discarded.

The sticks to be stained were placed in large-bore test tubes and inoculated with heavy spore suspensions of the respective fungi. The cultures thus prepared were incubated at laboratory temperatures for 30 days, at the end of which time almost all the specimens were stained throughout and ready for testing. The uninfected controls were tested promptly after sawing, or, in the case of tests involving heat treatment of the wood, they were placed in preliminary storage for 30 days in sterile, closed containers. A fuller description of the handling and staining procedures appears in the first report (*2*).

STAIN FUNGI USED

The fungi used and the species of wood from which they were solated are listed in table 1. *Ceratostomella pilifera* (Fr.) Wint. and *raphium rigidum* (Pers.) Sacc. are among the predominant species taining the sapwood of pine and red gum lumber, respectively.

TABLE 1.—*Test fungi and species of wood from which they were isolated*

Fungus	Culture No.	Original wood substrate
ratostomella pilifera	3	Shortleaf pine (*Pinus echinata* Mill.).
. pilifera	185	Longleaf pine (*P. palustris* Mill.).
pilifera	186	Loblolly pine (*P. taeda* L.).
pini	202	Shortleaf pine.
pini	212	Ponderosa pine (*P. ponderosa* Dougl.).
ips	157	Longleaf pine.
ips	202	Shortleaf pine.
aphium rigidum	198	Red gum (*Liquidambar styraciflua* L.).

C. pini Münch and *C. ips* Rumbold are primarily associated with stain accompanying certain beetle infestations of pine trees, but *C. ips* is also a common independent cause of stain in southern pine logs and lumber.

TESTING PROCEDURE

Tests were made of compression parallel to the grain, toughness, and, in some cases, of static bending. In addition, the specific gravity of each specimen was determined from its dimensions, weight, and moisture content when tested. Conventional machines were employed for the compression and bending tests and a special impact machine of the pendulum type, developed at the Forest Products Laboratory, was used for tests of toughness. In the bending and toughness tests loads were applied at the center of an 8-inch span. The compression tests were made on pieces 2½ inches long taken from the ends of the specimens used in the toughness tests but well removed from the zone of fracture.

ANALYSIS OF DATA

The effect of staining on the several wood properties is appraised on the basis of the geometric mean ratios of stained wood to control values. By virtue of the matching of stained specimens and controls in pairs, it is assumed that any differences between the members of a pair due to factors other than the blue stain were essentially equalized. A ratio is held to be significantly different from 1 if its logarithm is significantly different from 0.[5] (A mean value of 1, of course, would indicate no difference between the stained wood and the controls.)

PROPERTIES OF WOOD AS AFFECTED BY BLUE STAIN

TOUGHNESS AND WORK TO MAXIMUM LOAD

The results of the tests are summarized in table 2. Values indicating a reduction in a particular property that individually are mathematically significant on the basis stipulated above are marked by asterisks. Considering first the group of tests as a whole, the results are in good agreement with previous reports of similarly controlled experiments. Toughness and work to maximum load are both regarded as an index of shock resistance and were the only properties analyzed that were greatly affected. The greatest significant reduction in toughness amounted to about 75 percent and the smallest to about 9 percent. Similarly, the greatest significant reduction in work to maximum load amounted to about 41 percent and the smallest to about 14 percent. Had total work been included it presumably would have corresponded more closely to the toughness values, as was the case in earlier tests (*2*).

[5] Because there is no theoretical or known reason why any effect of blue stain fungi on the wood properties studied should be other than in the direction of a reduction, and any differences due to staining, therefore can be regarded as fixed with respect to sign, a 10-percent level of probability is taken as the basis for appraising significance rather than its equivalent — a probability of 5 percent without regard to the direction of the difference. The authority referred to for this procedure is Goulden (*6*).

TABLE 2.—*Specific gravity and strength of blue-stained pine sapwood*

TEST SPECIMENS NOT GIVEN STERILIZATION TREATMENT

Test No.	Stain fungus	Culture No.	Wood species	Number of matched pairs of test specimens	Average relative value for stained wood[1] (value for matched controls=100)				
					Specific gravity[2]	Compression parallel to grain	Static bending		Toughness
							Modulus of rupture	Work to maximum load	
5	*Ceratostomella pilifera*	185	*Pinus palustris*	12	98.8	104.4	100.0	*71.0	*55.1
6	do	185	do	18	*97.9	*93.8	*96.7	*74.9	*59.2
2	*Graphium rigidum*	198	*P. taeda*	16 to 18	98.5	97.9			94.4
3	do	198	*P. echinata*	14 to 16	98.4	*89.8	99.0	94.2	*75.2
4	do	198	*P. taeda*	24 to 25	100.8	100.9	*95.6	*75.7	*76.0

TEST SPECIMENS STEAMED 30 MINUTES AT 100° C.

Test No.	Stain fungus	Culture No.	Wood species	Number of matched pairs of test specimens	Specific gravity[2]	Compression parallel to grain	Modulus of rupture	Work to maximum load	Toughness
1	*C. ips*	157	*P. taeda*	19	100.0	102.4			*90.6
1	do	202	do	20	99.6	102.2			*52.9
1	*C. pilifera*	3	do	18	98.9	105.2			*63.1
1	do	186	do	20	99.3	110.3			*58.8
1	do	185	do	21	98.9	101.1			*29.4
5	do	185	*P. palustris*	12	99.3	111.2	99.1	*58.6	*36.8
6	do	185	do	18	*97.4	98.7	102.3	*64.6	*43.2
1	*C. pini*	202	*P. taeda*	20	99.4	105.6			*70.0
1	do	212	do	18	*98.6	109.6			*70.2
1	*G. rigidum*	198	do	21	99.3	99.5			*26.3
2	do	198	do	16 to 18	*96.2	*93.1			*24.6
3	do	198	*P. echinata*	14 to 16	101.8	*95.1	*90.0	*75.0	*76.5
4	do	198	*P. taeda*	24 to 25	*97.0	99.6	*95.6	*71.7	*57.3

TEST SPECIMENS STEAMED 5 MINUTES AT 12 POUNDS' PRESSURE

Test No.	Stain fungus	Culture No.	Wood species	Number of matched pairs of test specimens	Specific gravity[2]	Compression parallel to grain	Modulus of rupture	Work to maximum load	Toughness
2	*G. rigidum*	198	*P. taeda*	16 to 18	*96.9	*90.8			*24.4
3	do	198	*P. echinata*	14 to 16	100.0	101.8	*97.4	*85.6	*83.4

[1] Derived from the geometric means of the ratios: $\frac{\text{Value for control specimen}}{\text{Value for stained specimen}}$. Values that individually are significantly less than 100 are designated by asterisks (*). Certain values are significantly greater than 100, but as a result of moisture differences rather than the blue stain.
[2] Based on green volume and oven-dry weight.

SPECIFIC GRAVITY

In only 6 of the tests was the stain accompanied by a significant eduction in specific gravity. Nevertheless, in 16 tests out of the total f 20, the specific gravity of the stained wood was no less than 0.4 ercent lower than that of the controls and in most of these 16 cases was at least 1 percent lower. Mathematical analysis of the aggreate result [6] indicates that there was a definite tendency toward specific avity reduction by the several stain fungi. In the 6 tests where ecific gravity losses were individually significant, the greatest reducon was 3.8 percent and the smallest 1.4 percent.

COMPRESSION

The effect of staining on compression parallel to the grain was rather riable; in many cases an increase in this property was noted. However, significant increases occurred only in tests 1 and 5, in which a mber of the stained compression specimens were found at the time of

[6] According to Fisher's (5, p. 101), treatment of the combination of probabilities from tests of significance ggested to the writers by R. A. Chapman).

testing to be drier than those in the other tests, the moisture content being substantially less than in the controls and frequently near the fiber-saturation point. It is therefore assumed that moisture differences, rather than any effect of staining, were responsible for the significant increases in compression parallel to the grain.[7] The five significant reductions in compression strength all fell within a range of about 5 to 10 percent; hence were comparatively substantial. It should be noted, however, that these results were, with one exception, by the same fungus, and therefore are not necessarily representative of the group.

MODULUS OF RUPTURE

Individually significant reductions in modulus of rupture, ranging from about 3 to 10 percent, occurred in five of the nine tests of this property, and lesser, not individually significant, reductions occurred in two of the tests. As in the case of specific gravity, the aggregate result was a statistically significant reduction in this property.

SPECIFIC ACTION OF FUNGI

The four species of blue stain fungi did not affect the wood with equal severity, and the same fungus did not have the greatest effect on all properties. *Ceratostomella pilifera* reduced the toughness of the unheated wood and the work to maximum load of the heated wood appreciably more than did *Graphium rigidum*. On the other hand, *G. rigidum* had the greater effect in the case of modulus of rupture of the heated wood. Definitely assignable differences between the effects of these two fungi were not brought out by the other tests. *C. ips* and *C. pini*, although they produced a more intense stain than *C. pilifera* or *G. rigidum*, on the whole did not weaken the wood as much.

In line with some of the results previously reported, staining tended to have a greater effect on the heated wood than on the unheated. Differences in this respect were not evidenced in all properties, but mainly by work to maximum load and toughness. The tests were not replicated sufficiently to permit a quantitative analysis of the effects of heating.

DISCUSSION AND CONCLUSIONS

Reductions in compression and bending strength as great as some of those reported by Chapman (*2*) and again encountered in these tests appeared to be sufficiently unusual to warrant a special search for their cause. The answer apparently lies to a considerable extent in the respective abilities of the several fungi to extend their development beyond the wood rays, upon which their attack typically is centered, and to penetrate directly the walls of the tracheids. Microscopic examination disclosed that direct cell-wall penetration was common with *Graphium rigidum*, somewhat less so with all strains of *Ceratostomella pilifera*, and only occasional with the strains of *C. ips* and *C.*

[7] Losses of moisture from the test specimens took place largely during the incubation periods and, of course, were greatest from portions nearest the mouth of the culture tubes. It was for this reason that the stained compression specimens, which were taken near one end of the toughness specimens, tended to have a lower moisture content; the controls, having been tested immediately or else stored in closed containers, did not have the same opportunity to dry out as the stained pieces. Except for some of the compression specimens in tests 1 and 5, the moisture content of the pieces remained high enough not to interfere with the test results. An increase in the strength of wood as it dries does not occur until the moisture content falls approximately to the fiber-saturation point; practically speaking, the point where the cell walls are saturated but no free water is present in the cell cavities.

pini. An abundance of *G. rigidum* hyphae in the tracheids regularly penetrating the walls also offers a tenable explanation for reductions in specific gravity by this fungus amounting in one case to almost 4 percent. Findlay and Pettifor (*3*) have called attention to the fact that the reduction in specific gravity resulting strictly from a destruction of food materials stored in the wood rays probably would not exceed about 2 percent in Scots pine.

The microscopic examination further brought out what appeared to be a definite relation between the relative abundance of cell-wall perforations by a particular fungus and the toughness of the wood. This relationship extended in part to the different species of fungi but did not hold for *Ceratostomella pilifera* and *Graphium rigidum*. It is assumed, of course, that a large proportion of the reduction in toughness in all cases was accomplished by the typical operations of the fungi strictly within the ray cells, as was essentially the case with *C. ips* and *C. pini*. Further evidence for the correctness of this assumption is offered by the fact that certain decay fungi may markedly affect the toughness of wood without penetrating the walls of the wood fibers or tracheids (*1*, *11*).

In view of the results of the present study, it is rather surprising that *Graphium rigidum* plays a somewhat minor part in the staining of pine lumber whereas *Ceratostomella ips* is one of the predominant blue stain species. This leads to the generalization that predominance of a particular stain fungus in a certain kind of wood may not necessarily signify a greater local effect on the wood properties than that of a less prominent fungus. However, it is known that different strains of fungi may differ variously in their effect on wood. Hence the results with a comparatively few strains should not be interpreted too closely.

While the intensity of discoloration may be a criterion of the intensity of attack and amount of strength reduction caused by a single species of blue stain fungus, it apparently is not necessarily indicative for different species. As already mentioned, *Ceratostomella ips* and *C. pini* produced the darkest stain but otherwise affected the wood least. On the other hand, the extent of staining (i. e., amount of discolored wood) is a fairly reliable index of the magnitude of fungus attack and therefore might have some value as a basis for estimating relative strength losses, irrespective of the fungus or fungi concerned. For example, results obtained by Findlay and Pettifor, with naturally infected pinewood, indicated that loss in toughness was roughly proportional to the extent of staining. Specimens having 0 to 39 percent of the cross-sectional areas stained retained about 94 percent of their original toughness, whereas specimens with 70 to 100 percent of the cross-sectional area stained retained about 88 percent of their original toughness. However, with a little more than one-third of the cross-sectional area stained, about one-half of the maximum toughness reduction occurred. This result, if representative, possibly would place excessive demands on the accuracy of estimation.

In considering the practical aspects of blue stain, it should be kept in mind that the preceding results and those of the more recent previous investigations are based to a considerable extent on wood that had received some degree of heat sterilization. The factor of heating, as well as others introduced by laboratory experimentation, raises the question of how nearly results as obtained are representative of the

blue-stained wood ordinarily encountered. Greater strength losses in heated wood have been explained by assuming that the heat produced changes in the wood, rendering more of it immediately available to the fungi. However, with sufficient time and favorable conditions, the fungi themselves may effect some of the nutritionally significant changes created by mild heating, in which case the ultimate effects of staining in slowly air-dried wood might approach those found in the heated wood. The critical period for stain occurrence in air-dried lumber is generally believed not to exceed about 30 days in the majority of cases. Consequently, in air-seasoned board stock at least, strength reductions caused by blue stain are probably variously less than the tests on the steamed wood indicate. Chapman (*2*) reported that stained lumber naturally infected on the seasoning yard revealed less direct cell-wall penetration than existed in heated test specimens. Furthermore, he found that values for the wood stained by *Graphium rigidum* and *Ceratostomella pilifera*, in tests that incorporated a heat treatment consisting in all but one case of 30 minutes at 100° C., were lower than those in tests with no heat treatment by approximately the following percentages: Specific gravity, 0; modulus of rupture, 4; total work in bending, 30; toughness, 29.

Sufficient data have been accumulated to indicate rather definitely that blue stain, at least in unheated pinewood, reduces most strength properties comparatively little and need uot be a cause for discrimination from this standpoint except where strength, particularly shock resistance, is a prime requisite. Considering, necessarily somewhat subjectively, the results of the present and prior experiments, including those by others, the authors believe that fully developed blue stain in naturally infected pinewood commonly may reduce specific gravity 1 to 2 percent, strength in compression parallel to the grain and modulus of rupture 1 to 4 or 5 percent, and toughness 15 to 30 percent. Surface hardness may be reduced 2 to 10 percent (*3*, *7*). The duration of favorable conditions for staining and the particular fungus or fungi involved apparently are the most important factors determining the extent of weakening.

Exceptions to these conclusions may be found in certain woods other than pine, as for example, in obeche wood studied by Findlay and Pettifor (*4*). Obeche specimens stained by *Botryodiplodia theobromae* lost as much as 43 percent of toughness, 19 percent of bending strength, and 12 percent of specific gravity.

It may be significant that, with a reduction in specific gravity of about 2 percent, the corresponding reduction in strength caused by blue stain fungi does not appear to be a great deal less than that caused by a vigorously attacking decay fungus; in fact, there is substantial evidence that the toughness reduction may be even greater in some cases. For example, in laboratory decay tests by one of the authors (*11*), conducted in practically the same manner as the present stain study, the average percentage losses in strength associated with a 2-percent reduction in the specific gravity of red gum infected with *Polyporus versicolor* were about as follows: Compression parallel to grain, 7; modulus of rupture, 10; toughness (total work), 35–40; and hardness, 7.

To complete the discussion of the practical aspects of blue stain it should be pointed out that, in general, the conditions that favor the development of stain fungi also favor the development of decay

fungi; hence it is often desirable to examine heavily stained materia for signs of decay. Important decay infection does not necessaril accompany heavy and extensive blue stain, since blue stain typicall precedes decay, but that it commonly may do so is suggested by a recent report (*15*) in which the strength of heavily stained lumber was compared with that of comparably seasoned lumber that had beer kept bright by chemical treatment. Reductions in the strength of the stained lumber in some cases were greater than could be accountec for by the presence of the stain alone.

SUMMARY

The effect of blue stain on the specific gravity and strength of pine wood artificially inoculated with pure cultures of four species of fungi and with different strains of some of them, was essentially the same as reported for prior, similarly controlled studies. Although all strength properties appeared to be lowered generally, only toughness was affected to an extent of general practical significance.

The different species of blue stain fungi did not affect the wood with equal severity, nor was their relative order of superiority in this respect entirely the same for all properties tested. There was some evidence of a broad relation between the abundance of direct cell-wall penetration by the fungi and reduction in toughness. The intensity of the discoloration caused by the different fungi was not indicative of the severity of attack nor of the comparative weakening caused by each. The frequency of association between a particular blue stain fungus and a certain kind of wood apparently does not necessarily indicate the inherent ability of the fungus to attack the wood and to affect its strength.

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THE IMPAIRMENT IN NUTRITIVE VALUE OF CORN GRAIN DAMAGED BY SPECIFIC FUNGI [1]

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INTRODUCTION

It has been estimated (*6*) [2] that the corn crop of the State of Illinois suffers an average annual loss of at least 20 percent from diseases of all kinds. The total loss from ear rot is assessed at 7.5 percent, equivalent in terms of annual production to 23 million bushels. In this estimate rot-damaged kernels were considered as a loss.

The extent of infection of the corn grain by fungi of various kinds is an important item in the grading of corn either by physical or chemical means (*12*) or by performance tests (*3*). However, in view of the fact that the preponderant proportion of the corn crop in this country is used as animal feed, the extent to which a crop should be penalized for its content of unsound corn should depend upon the impairment in nutrituve value that has resulted from the type of damage incurred.

Moldy corn may be toxic to pigs (*5*) and horses (*9*), and, possibly, in large quantities, to chickens (*8*), but the investigations referred to cannot be taken to mean that all moldy corn is toxic, or that any particular mold will produce a toxic grain, because unfortunately the organism responsible for the toxicity, whether fungus or bacterium, was not identified. In other experiments (*1, 11*), molds of the genus *Fusarium* have been definitely and causally related to the toxicity of infected corn.

Insofar as the writers are aware, no experiments on the nutritive value of unsound as compared with sound corn have been reported in the literature. Hence, at the suggestion of Dr. George H. Dungan of the Agronomy Department of the University of Illinois, the experiments described herein were undertaken, having as their object the *determination of the effect on the protein and energy values of the corn kernel of infection with specific fungi.* The fungi chosen for study were *Diplodia zeae* (Schw.) Lev., *Fusarium moniliforme* Sheldon, and *Gibberella zeae* (Schw.) Petch [*G. saubinetii* (Mont.) Sacc.]. These fungi are arranged in the order of the seriousness of the damage they produce on the corn crop in Illinois (*6*), although at times and in certain sections of the State, fusarium ear rot has accounted for significant damage to more than 25 percent of the ears.

EXPERIMENTAL MATERIALS [3] AND METHODS

The ears of corn (*Zea mays* L.) were inoculated by hand with pure spores of the organisms. When harvested, the kernels shelled from the ears infected with the diplodia and gibberella organisms were

[1] Received for publication December 22, 1939.
[2] Italic numbers in parentheses refer to Literature Cited, p. 140.
[3] The material used in these experiments was provided through the generous cooperation of Dr. Benjamin Koehler of the Department of Agronomy.

damaged to the extent of 94 to 98 percent in the former case, but only 53 percent in the latter. In the case of the fusarium-damaged corn, because of the spotted nature of the infection, the damaged kernels were separated by hand. For each sample of damaged corn, a sample of sound corn exhibiting a very inappreciable damage from mold was secured for comparative purposes from the same field and crop. All samples were analyzed chemically and their heat of combustion determined in the bomb calorimeter. The results are compiled in table 1.

TABLE 1.—*Varieties, chemical composition, and gross energy value of sound and fungus-damaged corn samples tested*

Variety[1]	Condition	Weight of 100 kernels	Moisture	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Gross energy per gram	Ash	Phosphorus	Calcium
		Grams	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Small calories*	*Percent*	*Percent*	*Percent*
Illinois hybrid 947	Sound	23.828	9.72	11.12	4.10	1.84	71.82	4,093	1.40	0.328	------
	Diplodia rot	20.046	10.06	11.69	2.64	2.51	71.58	4,039	1.52	.350	------
Illinois hybrid 92	Sound	25.085	9.54	10.88	4.00	1.76	72.43	4,082	1.39	.323	0.0050
	Diplodia rot	21.370	9.58	11.19	3.08	2.52	72.10	4,040	1.53	.330	.0061
Illinois hybrid 172	Sound	23.605	9.78	10.75	4.26	1.74	72.10	4,084	1.37	.311	------
	Diplodia rot	21.569	10.46	11.00	3.12	2.31	71.48	4,003	1.63	------	------
Iowa hybrid 13	Sound	31.139	9.81	10.94	3.74	1.78	72.29	4,047	1.44	------	------
	Diplodia rot	23.709	10.22	11.69	2.60	2.26	71.67	4,005	1.56	------	------
Illinois hybrid 960	Sound	26,294	8.96	7.69	4.46	1.92	75,69	4,019	1.28	.276	.0033
	Diplodia rot	21.522	8.83	7.62	2.82	2.46	76.89	3,962	1.38	.308	.0060
Improved Reid Yellow Dent.	Sound	32.590	8.84	8.88	4.65	1.91	74.37	4,060	1.35	.312	.0023
	Diplodia rot	25.874	8.90	9.12	3.06	2.50	74.97	3,994	1.45	.318	.0043
Do	Sound	23.546	9.72	10.62	4.30	1.72	72.34	4,077	1.30	.303	.0060
	Diplodia rot	22.664	9.60	12.19	2.90	2.64	71.06	4,055	1.61	.366	.0072
	Gibberella rot	17.414	9.56	13.00	3.94	2.94	68.71	4,113	1.85	.385	.0078
Do	Sound	30.268	7.46	9.00	4.31	1.98	75.81	4,127	1.44	.228	.0038
	Fusarium rot	20.835	8.46	9.88	3.57	2.60	73.87	4,064	1.62	.258	.0087

[1] The hybrids had the following pedigrees: Illinois hybrid 947, (R4 × Pr) × Tr × 317); Illinois hybrid 92, (WF9 × 5120)×(R4 × Hy); Illinois hybrid, 172, (R4 × Hy)×(A × 540); Illinois hybrid 960, (R4 × Hy)×(317 × 701); Iowa hybrid 13, (349 × 317)×(345 × 401). The improved Reid Yellow Dent was the Illinois Agricultural Experiment Station strain of this variety.

For the protein tests, three rations were made up for each pair of corn samples tested. Two of the rations contained 88 and 87 percent of the sound and unsound corn, respectively, together with vitamin and mineral supplements. The lower oil content of the damaged corn was compensated for by the inclusion of 1 percent of corn oil in the corresponding ration so that the gross energy values would be approximately equal. These rations contained only from 9.5 to 12.5 percent of crude protein, mostly derived from the comparative corn samples, but were otherwise complete. The third, or diluting, ration contained no corn at all, but the same mineral and vitamin supplements as the other two, and enough starch, sucrose, and corn oil to furnish approximately the same gross energy per gram.

The rations for the energy tests contained 68 and 67 percent of sound and unsound corn, respectively, and were supplemented, not only with vitamins and minerals, but also with 20 percent of casein. They were, therefore, nutritively complete, closely similar in gross energy value by actual determination with the bomb calorimeter, and possibly different only in the extent of utilization of the contained energy. The composition of the rations is shown in table 2.

The paired-feeding technique was used throughout these experiments. In the energy tests, paired rats were fed equal amounts of food and the relative energy values were judged by the gains in body weight produced and by the gross energy content of the carcasses of pair mates. In the protein tests, considerable differences in live weight of paired rats were not allowed to develop. As soon as a tendency for a difference in weight appeared, the ration of the rat gaining the faster was diluted with the diluting ration above described, containing only about 0.5 percent of nitrogen, to the extent required to maintain equality in weight between pair mates. Differences in protein quality of comparable rations were, under these conditions, inversely proportional to the amounts of dietary protein required for equal gains in body weight.

The rats were started generally at weights varying between 40 and 55 gm. The digestibility of dietary nitrogen or of dietary energy was determined in each experiment in a 7-day collection period on constant intake of food, a marker (F_2O_3) being used to separate the feces. At the termination of the feeding periods, which lasted usually for 6 to 12 weeks depending upon the rates of growth secured, the rats were killed with ether, the body lengths from tip of nose to root of tail measured, and the empty body weights determined. In one experiment (No. 168) concerned with the energy value of sound and fusarium-infected corn, the carcasses of the experimental rats were frozen, ground finely in a hand mill, thoroughly mixed, and the gross energy (heat of combustion) of weighed portions determined in the bomb calorimeter.

TABLE 2.—*Composition of the experimental rations used in the protein and the energy studies with sound and fungus-damaged corn*

Ingredients	Protein studies			Energy studies	
	Sound corn ration	Unsound corn ration	Diluting ration	Sound corn ration	Unsound corn ration
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
Sound corn	88.0	0.0	0	68.0	0
Unsound corn	0	87.0	0	0	67.0
Salt mixture	4.0	4.0	4.0	4.0	4.0
Dried yeast	6.0	6.0	6.0	6.0	6.0
Cod-liver oil	1.5	1.5	1.5	1.5	1.5
Wheat-germ oil	.5	.5	.5	.5	.5
Corn oil	0	1.0	4.0	0	1.0
Starch	0	0	74.0	0	0
Sucrose	0	0	10.0	0	0
Casein	0	0	0	20.0	20.0

EXPERIMENTAL RESULTS

The analyses of the samples of sound and unsound corn, collected in table 1, together with the weights of 100 kernels in each case, reveal some fairly consistent differences. In all comparable samples, the mold-damaged corn contained less fat, averaging about 25 percent less, than the sound corn, more ash, calcium, and phosphorus, distinctly more crude fiber, and generally more nitrogen. These differences must result from the destructive action of the mold on some of the nutrients in the corn and from the addition of the nutrients of the mold itself. The germ seems to be the most vulnerable part of the kernel for most

types of damage; of the constituents of the germ, it has been shown by Rabak (*7*) and Zeleny and Coleman (*12*) that the oil is definitely destroyed by molds. In fact, Zeleny and Coleman found that fat acidity is a satisfactory chemical measure of soundness in corn, being more reliable even than the percentage of damaged kernels. The lower content of fat (ether extract) in the damaged corn than in the sound corn can probably be explained, therefore, on the basis of a direct destruction of fat by the fungus. The increased percentage content of protein (N×6.25), crude fiber, and ash in the damaged corn may be in part a mathematical resultant of the lowered percentage of fat in the germ and in part a distortion of the proportions normally existing among the anatomical parts of the corn kernel. These increases are analogous to the increased content of carotenoids that Clark and Gring (*2*) observed in damaged corn, though in the latter case a possible differential effect of storage for more than a year on the sound and the damaged grain must also be considered.

TABLE 3.—*Average results of the protein studies when sound and fungus-damaged samples of different varieties of corn were fed to rats*

Experiment No.	Variety of corn	Condition of corn	Pairs of rats used	Average time on feed	Average total gain in weight	Average body length	Digestibility of nitrogen[1]: Average	Digestibility of nitrogen[1]: Pairs conforming	Ratio of nitrogen intake[1]: Average	Ratio of nitrogen intake[1]: Pairs conforming
			Number	*Days*	*Grams*	*Millimeters*	*Percent*	*Number*		*Number*
124	Illinois hybrid 92	Sound	4	48.5	57.6	172.8	84.2	3	1	4
		Diplodia rot			56.4	171.8	82.5		1.22	
162	Improved Reid Yellow Dent.	Sound	6	38.8	40.5	169.2	83.5	5	1	6
		Diplodia rot			39.3	170.5	81.8		1.34	
139	Iowa hybrid 13	Sound	9	69.2	60.8	177.5	85.1	8(8)	1	8(8)
		Diplodia rot			61.7	177.2	81.9		1.18	
143	Illinois hybrid 947	Sound	10	65.9	62.5	178.3	84.1	7(8)	1	10
		Diplodia rot			61.6	177.0	80.6		1.27	
144	Illinois hybrid 172	Sound	8	71.9	71.1	179.6	84.2	7.5	1	7(7)
		Diplodia rot			69.6	179.7	82.6		1.25	
206	*Improved Reid Yellow* Dent.	*Sound*	10	70.6	*85.1*	*185.9*	*82.8*	8(9)	1	10
		Diplodia rot			84.4	185.4	79.6		1.25	
208	Illinois hybrid 900	Sound	10	71.2	67.4	175.2	81.6	8	1	8
		Diplodia rot			66.6	176.2	79.4		1.13	
119	Improved Reid Yellow Dent.	Sound	8	5.6	−12.4					
		Gibberella rot.			−15.1					
167	do	Sound	10	46.5	18.1	155.0	81.9	10	1	10
		Fusarium rot			17.1	151.3	75.0		1.34	

[1] When the number of determinations of digestibility and of the ratio of nitrogen intakes required for equal gains is less than the number of pairs of rats originally placed on experiment (column 4), the reduced number is indicated in parentheses.

The average results of the protein studies are collected in table 3. The statistical significance of the differences between averages is indicated by the "number of pairs conforming," given in columns 9 and 11. Thus, in experiment 124, of the four pairs of rats used, a greater digestibility of nitrogen was observed in three pairs, and a smaller intake of nitrogen required in all four pairs by the rat on the sound corn ration. In this experiment, 1 gm. of nitrogen in the sound corn proved to be the nutritive equivalent of 1.22 gm. of nitrogen in the diplodia-rotted grain.

The evidence in table 3 demonstrates that the diplodia fungus definitely, if only slightly (about 2.5 percent), depressed the digestibility of protein in the corn kernel and lowered its biological value. On an average, 1 gm. of nitrogen in the sound corn was the equivalent of 1.23 gm. of nitrogen in the diplodia-damaged corn, a difference that cannot be accounted for, except in small part, by the impairment in digestibility observed. This difference indicates that the nitrogen in the damaged corn was only 81.3 percent $[(1 \div 1.23) \times 100]$ as valuable to the growing rat as the nitrogen in the sound corn.

The fusarium-damaged corn showed a greater impairment in the digestibility and in the total nutritive value of its nitrogen for maintenance and growth than the diplodia-damaged corn. The depression in digestibility averaged 6.9 percent; and in the promotion of growth, 1 gm. of nitrogen in the sound corn was the nutritive equivalent of 1.34 gm. of nitrogen in the damaged corn. This means that the nitrogen of the damaged corn was only 74.7 percent as valuable to the growing rat as the nitrogen in the sound corn. However, it cannot be inferred that fusarium rot is more deleterious to the nutritive value of corn than diplodia rot, because in the particular samples tested the extent of damage may not have been comparable.

The protein damage occasioned by the diplodia and fusarium fungi may be entirely the result of the conversion of the proteins of the corn kernel into mold proteins, which have been shown to be of very low biological value (*4*, *10*). This explanation is offered on the assumption that all molds are similar in the nutritive value of their proteins for the support of mammalian growth, the references cited having to do with other molds than those here studied.

The gibberella-damaged corn proved to be so toxic for rats that too ittle was eaten to permit a fair test. However, even with this limited 'onsumption of the ration containing the damaged corn, many of the 'ats died.

TABLE 4.—*Average results of the energy studies when sound and fungus-damaged samples of different varieties of corn were fed to rats*

Experiment No.	Variety of corn	Condition of corn	Pairs of rats used	Average time on feed	Gain in body weight: Average	Gain in body weight: Pairs conforming [1]	Body length: Average	Body length: Pairs conforming [1]	Digestibility of energy: Average	Digestibility of energy: Pairs conforming [1]
			Number	*Days*	*Grams*	*Number*	*Millimeters*	*Number*	*Percent*	*Number*
157	Illinois hybrid 947	Sound Diplodia rot	10	66.2	141.5 133.6	9	199.2 200.2	6.5	90.2 88.9	10
205	Improved Reid Yellow Dent	Sound Diplodia rot	10	84	149.9 143.1	8.5	202.0 201.9	6.5	90.5 89.2	10
207	Illinois hybrid 960	Sound Diplodia rot	10	77	156.7 148.8	9.5	204.5 205.1	5	91.4 90.4	9
168	Improved Reid Yellow Dent	Sound Fusarium rot	10	56	57.2 46.4	10	175.8 168.4	10	91.0 88.9	10

[1] See text for further explanation

The average results of the energy studies on diplodia-damaged and 'usarium-damaged corn are given in table 4. Again the evidence of ıutritive damage is clear-cut. The gains in body weight and the

digestibility of energy were definitely depressed on the rations containing unsound corn as compared with the rations containing sound corn. Here, also, the damage associated with fusarium infection was definitely greater than that associated with diplodia infection.

To complete the evidence with reference to impairment of energy utilization, the rats in experiment 168 were killed and the energy content of their carcasses determined, with the results assembled in table 5. In all 10 pairs the rat on the unsound corn contained less gross energy than the rat on the sound corn, the average deficit amounting to 25 calories, or 13 percent.

TABLE 5.—*The energy content of the rats used in experiment 168*

Pair No.	Energy content of carcass in—			
	Calories per gram [1] of rats fed—		Total calories of rats fed—	
	Sound corn ration	Unsound corn ration	Sound corn ration	Unsound corn ration
	Calories	*Calories*	*Calories*	*Calories*
1	1.93	2.00	179	174
2	1.99	1.83	181	137
3	1.90	1.81	145	122
4	1.96	1.91	218	163
5	2.03	1.70	219	159
6	2.09	1.97	236	220
7	1.86	2.07	186	184
8	1.68	1.91	197	197
9	1.97	2.03	164	156
10	2.06	1.74	202	151
Average	1.95	1.90	193	168

[1] Calculated on empty weight of rats.

CONCLUSIONS

Infection of corn with diplodia ear rot (*Diplodia zeae* (Schw.) Lév.) or fusarium ear rot (*Fusarium moniliforme* Sheldon) definitely impairs the digestibility of the contained nitrogen and energy and the nutritive value of the protein and energy for the promotion of growth in the rat. The damage done by the fusarium organism may be more severe than the damage done by the diplodia organism.

The chemical composition of corn undergoes definite changes as the result of infection with these molds, prominent among which is a marked loss in ether-extractable constituents.

A sample of corn containing as much as 53 percent of kernels damaged by *Gibberella zeae* (Schw.) Petch. [*G. saubinetii* (Mont.) Sacc.] is extremely toxic to young albino rats.

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PEA SEED TREATMENT WITH CHEMICAL DUSTS [1]

By J. C. WALKER, *professor of plant pathology*, E. J. DELWICHE, *professor of agronomy*, and PAUL G. SMITH, *assistant in plant pathology and agronomy, Wisconsin Agricultural Experiment Station* [2]

INTRODUCTION

Among the first applications of organic mercury dusts to pea seed were those made by Jones (*7*)[3] for the control of seed-borne pathogens, particularly *Ascochyta* spp. It became evident from these investigations that the protection of the seed from decay by organisms in the soil was also an important phase of treatment. The value of certain organic mercury compounds for this purpose was demonstrated by the same investigator (*8*) and by Haenseler (*4*). The former found that germination was reduced markedly when the soil was watered heavily immediately after sowing, particularly with the wrinkled, seeded varieties with sugary cotyledons, as compared with smooth-seeded varieties with starchy cotyledons. Seed treatment offset to a certain degree the detrimental effect of high soil moisture, and in the case of the smooth-seeded variety, Alaska, a good stand was secured with treated seed regardless of the soil moisture and soil temperature. Hull (*6*), in England, noted that high soil moisture reduced emergence owing to the activity of soil organisms on the seeds. He also found that the smooth-seeded varieties, Pilot and Alaska, were less liable to injury than Gradus, a wrinkle-seeded variety, and that organic mercury dusts were helpful in increasing emergence. Brett et al. (*2*), also in England, found seed treatment beneficial to the stand in early-spring sowings, the benefits decreasing as the sowings were delayed. A study in the same country by Padwick (*10*) of the causal agents of pea-seed rotting in the soil showed that a large number of organisms not otherwise pathogenic on peas were involved in this complex disease and that the foot rot organisms such as *Ascochyta pisi* Lib. and *Mycosphaerella pinodes* (Berk. & Blox.) Stone were apparently of little importance in seed rotting.

Following the earlier use of organic mercury dusts other materials were tested. Horsfall (*5*) introduced the use of red copper oxide (Cu_2O), particularly for the variety Surprise. This compound was recommended by Anderson et al. (*1*) in Illinois, by Cook (*3*) in Virginia, and by Ogilvie and Hickman (*9*) in England. Zinc oxide when tried by Cook (*3*) tended to stunt the plants after germination, while Ogilvie and Hickman (*9*) reported it to be less effective than red copper oxide.

The present paper is a report of seed-treatment experiments with canning peas (*Pisum sativum* L.) in Wisconsin during a 4-year period from 1936 to 1939, inclusive, particularly with red copper oxide and 2-percent Ceresan.

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[2] The writers are indebted to Walter Virgin, William Carlsen, John McLean, and Lawrence Plzak, all of whom have assisted in certain phases of this work.

[3] Italic numbers in parentheses refer to Literature Cited, p. 146.

METHODS AND MATERIALS

During 1936 and 1937 tests were carried out on Colby silt loam a the branch experiment station at Marshfield, Wis. Seed was duste with red copper oxide and graphite according to manufacturers recommendations and planted in a grain drill in strips 4 feet wide an 16 rods long. Three plots of each treatment were planted on May 15 1936; two plots of each on May 25, 1937. Stand estimates were mad about 4 weeks after planting by taking the counts of plants in square yard areas at several random locations in each plot.

In 1938 and 1939, single-row plots of 110 or 150 seeds per row wer used. The individual row lots were dusted in separate packets an planted at approximately 10 seeds per foot of row. At the time c planting, the hands were dusted with the material used on the see in order to avoid removal of dust from the seeds during the plantin operation. All excess dust was left in each envelope. Four row plots of each lot of seed were used for each treatment arranged i random order. Counts of the complete stand in each were made abou 4 weeks after planting. In addition to the Marshfield tests in 193 (sown May 2), the same plot design was repeated in an early (April 25 and a late (June 3) planting on Miami silt loam at the branch experi ment station at Sturgeon Bay, Wis., and in an early planting (May 3 on the same soil type on the university farm at Madison, Wis. I 1939 plantings were made on May 9 at Marshfield, on the same dat at Sturgeon Bay, and on May 13 at Madison.

The pea seed used was secured from the branch experiment statio at Sturgeon Bay. The red copper oxide was provided by Röhm an Haas Co. The Ceresan contained 2 percent of ethyl mercuric chlorid and was provided by the Bayer-Semesan Co.

For comparative purposes the rainfall record was used for th period beginning 3 days before sowing and extending for 11 days be yond sowing for each location. Significance of difference in all case was checked by the analysis of variance method.

EXPERIMENTAL RESULTS

The stand counts at Marshfield for 1936 and 1937 are given i table 1. At this location no significant increase in stand was secure with either the smooth-seeded Alaska or the four wrinkle-seede varieties. No detrimental effect was apparent except a temporar slight retardation in vine growth in 1937 with all varieties excep Wisconsin Penin. The soil moisture at the time of sowing was lowes in 1937.

In table 2 are the stand counts from all plots in 1938 and 1939 It will be noted that two additional locations were added. Again n benefits in stand were secured in 1938 with the Alaska seed from eithe treatment. At Madison the only significant increase was with re copper oxide on one variety, Wisconsin Perfection. At Marshfiel the oxide was beneficial on all three wrinkle-seeded varieties, wherea with Ceresan no significant increases in stand were secured. At Sturgeon Bay significant increases were noted at both planting dates with each compound in all wrinkle-seeded varieties except Merit. In a majority of cases the increase in stand was greater with red copper oxide than with Ceresan. There was no clear-cut correlation between soil moisture as indicated by the precipitation records and the effectiveness of treatment.

TABLE 1.—*The effect of red copper oxide treatment on stand of pea varieties planted at the Marshfield Experiment Station, 1936 and 1937*

Variety	Year of test	Rainfall[1]	Stand counts (plants per square yard): Untreated	Stand counts: Treated	Stand counts: Difference required for significance between treatments (1:19)
		Inches	*Number*	*Number*	
Alaska	1936	1.32	83.8	81.3	10.8
Alaska	1937	.56	99.8	90.0	24.0
Wisconsin Early Sweet	1936	1.32	130.5	125.8	10.8
Surprise	1937	.56	106.8	103.8	24.0
Wisconsin Perfection	1936	1.32	72.2	77.9	10.8
Wisconsin Penin	1937	.56	73.0	74.0	24.0

[1] For the period inclusive of 3 days before and 11 days after sowing.

TABLE 2.—*The effect of seed treatment on stand of pea varieties in 1938 and 1939*

1938 EXPERIMENTS

Variety	Location of trial	Date of sowing	Rainfall[1]	Stand in the various treatments: Untreated	Stand: 2-percent Ceresan	Stand: Red copper oxide	Difference required for significance (1:19)	Significant (+) or insignificant (−) increase over untreated: Ceresan	Significant (+) or insignificant (−) increase over untreated: CuO
			Inches	*Percent*	*Percent*	*Percent*	*Percent*		
Alaska	Marshfield	May 2	2.51	78.7	76.0	78.0	7.3	−	−
	Madison	May 3	.66	97.3	98.0	98.7	6.0	−	−
	Sturgeon Bay	Apr. 25	2.34	90.0	91.8	92.7	6.4	−	−
	do	June 3	.44	85.5	83.6	91.8	7.3	−	−
Wisconsin Early Sweet.	Marshfield	May 2	2.51	58.0	57.3	65.3	7.3	−	+
	Madison	May 3	.66	91.3	92.7	96.0	6.0	−	−
	Sturgeon Bay	Apr. 25	2.34	70.0	80.0	90.0	6.4	+	+
	do	June 3	.44	55.5	78.2	89.1	7.3	+	+
Wisconsin Perfection	Marshfield	May 2	2.51	32.7	38.0	44.0	7.3	−	+
	Madison	May 3	.66	65.3	68.0	74.0	6.0	−	+
	Sturgeon Bay	Apr. 25	2.34	40.0	54.5	65.5	6.4	+	+
	do	June 3	.44	39.1	67.3	62.7	7.3	+	+
Wisconsin Penin	Marshfield	May 2	2.51	57.3	62.7	66.7	7.3	−	+
	Madison	May 3	.66	85.3	86.7	88.0	6.0	−	−
	Sturgeon Bay	Apr. 25	2.34	63.6	74.5	79.1	6.4	+	+
	do	June 3	.44	66.4	74.5	80.9	7.3	+	+
Merit	Sturgeon Bay	June 3	.44	81.8	87.3	89.3	7.3	−	+
Canner King	do	do	.44	67.3	79.1	85.5	7.3	+	+
Surprise	do	do	.44	46.4	65.5	75.5	7.3	+	+

1939 EXPERIMENTS

Variety	Location of trial	Date of sowing	Rainfall[1]	Untreated	2-percent Ceresan	Red copper oxide	Difference required for significance (1:19)	Ceresan	CuO
Alaska	Marshfield	May 9	.00	95.2	98.5	97.3	5.0	−	−
	Madison	May 13	.20	91.9	95.9	90.7	8.0	−	−
	Sturgeon Bay	May 9	1.26	95.2	97.9	98.0	9.3	−	−
Wisconsin Early Sweet.	Marshfield	May 9	.25	91.0	91.9	93.2	5.0	−	−
	Madison	May 13	.20	85.3	82.5	85.2	8.0	−	−
	Sturgeon Bay	May 9	1.26	33.9	55.3	93.5	9.3	+	+
Wisconsin Perfection	Marshfield	May 9	.25	82.7	80.9	81.9	5.0	−	−
	Madison	May 13	.20	84.9	87.3	90.0	8.0	−	−
	Sturgeon Bay	May 9	1.26	65.2	72.5	87.0	9.3	−	+
Wisconsin Penin	Marshfield	May 9	.25	73.3	78.3	75.9	5.0	+	−
	Madison	May 13	.20	79.3	77.9	77.7	8.0	−	−
	Sturgeon Bay	May 9	1.26	57.9	61.7	83.5	9.3	−	+

[1] For the period inclusive of 3 days before and 11 days after sowing.

In 1939 the two treatments were again used at each of the three locations of 1938. At Marshfield and Madison the season was very dry at the time of sowing. No increases of consequence, with one exception, were secured at either of these stations (table 2). At Sturgeon Bay the Alaska was again not influenced by treatment, while red copper oxide caused significant increases in stand for the three wrinkle-seeded varieties. The Ceresan treatment was effective in improving the stand of only one variety at Sturgeon Bay—Wisconsin Early Sweet—but in this case its effect was much less than that of red copper oxide.

DISCUSSION AND SUMMARY

The results of 4 years' trials with treatment of pea seed in Wisconsin show clearly that the benefits to be derived will vary with the season and location. All the fields used had been subjected to rather intensive pea production previous to the experiments. In accord with the findings already cited from England, the conditions which bring about stand reduction of sugary-cotyledoned forms are not detrimental to the starch-seeded Alaska, and consequently seed treatment was of no value to this variety at any time during the 4-year period.

In the soil of relatively high water-holding capacity at Marshfield significant increases from treatment were secured in only 1 out of 4 years. This was in the year of heaviest precipitation at that station during the planting period. It is important to note, however, that in that season (1938) only red copper oxide was beneficial and the increases in stand, although significant, were decidedly lower than those of the same seed samples in treated plots at other locations. It is obvious that at Marshfield, in this season, treatment did not correct adequately the "poor stand" difficulty.

The Sturgeon Bay soil, although of the same classification as that of Madison, benefited more consistently from pea-seed treatment than did the latter. In the early plantings of 1938 and 1939 it was in each case the moister soil. The early and late sowings at Sturgeon Bay in 1938, however, both showed benefits of treatment while the rainfall during the first sowing period was 2.34 inches as compared with 0.44 inch during the second.

It may be said, therefore, that the conditions which favor cotyledon decay during germination occur periodically but not necessarily consistently under average Wisconsin conditions. They do not seem to affect the stand of starchy-cotyledoned forms, but may reduce the stands of the sugary-seeded varieties. Red copper oxide is a safe and beneficial protective treatment for all varieties of the latter group. Ceresan, while sometimes beneficial, was not so effective as the oxide in these trials.

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THE EFFECT OF SOME MINERAL NUTRIENTS ON THE DEVELOPMENT OF CLUBROOT OF CRUCIFERS [1]

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INTRODUCTION

Many workers (*2, 3, 7, 10, 11, 12, 13, 15, 16*)[3] have demonstrated that varying the nutrition of a plant has a decided effect on disease development. In the majority of these studies nitrogen, potassium, and phosphorus were the elements under investigation. The specific response appeared to vary with the degree of plant resistance, with the parasite involved, and with the nutrients supplied. Petri (*7*) observed that, in general, nitrogenous dressings aggravate susceptibility, whereas phosphatic and potassic applications reduce it. In regard to the variety or strain of the host the findings of Gassner and Hassebrauk (*3*) with several rusts of wheat, barley, and oats illustrate the general case. Partially resistant hosts proved to be the most suitable material for nutrititional experiments. If the plants were either highly resistant or immune, varying the proportion and amount of salts in the culture solution did not change the disease reaction, whereas if extremely susceptible plants were employed the disease attack was so severe that the nutrititional effects were masked.

The possible influence of sulfur oils in cruciferous plants upon the development of clubroot (*Plasmodiophora brassicae* Wor.) was first suggested by Rochlin (*9*). The purpose of the present investigation was to vary the oil content of the host through controlled nutrition and to study the consequent effect upon the development of the disease. The mustard oils present in crucifers all contain sulfur and nitrogen while their glycosides also contain potassium. These nutrient elements were selected for study.

METHODS

In these experiments Shogoin, Cowhorn, and Purple Top Milan varieties of turnip (*Brassica rapa* L.), two strains of black mustard (*B. nigra* Koch) and Jersey Queen variety of cabbage (*B. oleracea* var. *capitata* L.) were used. The average percentages of clubroot-infected plants from several greenhouse trials of the above lots of crucifers

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[3] Italic numbers in parentheses refer to Literature Cited, p. 159.

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grown on naturally infested soil collected in southern Wisconsin are as follows:

	Percent of plants diseased
Strain No. 14 black mustard	85
Strain No. 10 black mustard	5
Shogoin turnip	100
Cowhorn turnip	40
Purple Top Milan turnip	0
Jersey Queen cabbage	100

As already pointed out (*18*) it is essential in greenhouse tests with clubroot to keep the soil reaction fairly acid; the soil in these tests was maintained at pH 6.0 to 6.5.

The foregoing forms were selected from collections previously made by Walker (*17*, *18*) as representing highly resistant (No. 10) and highly susceptible (No. 14) strains of black mustard, and completely susceptible (Shogoin) and completely immune (Purple Top Milan) varieties of turnip. The Cowhorn variety of turnip was selected because it consistently showed an intermediate percentage of infected plants and was undoubtedly heterozygous for the respective characters which had been completely or almost completely fixed in the other two varieties. Jersey Queen cabbage is representative of another form, all varieties of which are very susceptible to *Plasmodiophora brassicae*.

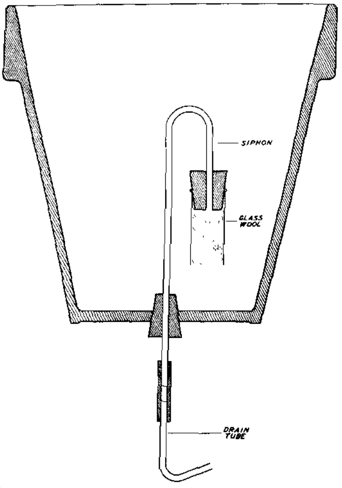

FIGURE 1.—Siphon drain for sand-culture pots. The bend in the 5-mm.-bore glass tubing is 2½ inches below the top of the pot. The 2-inch piece of 20-mm. glass tubing filled with glass wool serves to prevent sand from clogging the drain.

Heavily varnished (*6*), 8-inch clay pots were equipped with a modification of Tharp's automatic siphon (*14*) (fig. 1) which allowed the sand to become saturated about every 36 hours under a supply system to be described later. These pots were filled to within one-half of an inch of the top with fine white silica sand which had been washed several times in hot water and rinsed three or four times with distilled water.

Nutrients were supplied by a modification of the continuous-flow apparatus described by Allison and Shive (*1*). From 10-gallon reservoirs solutions flowed by siphon and gravity through 12-mm.

tubing to a 12-inch length of 0.3-mm.-bore capillary tubing, which was found to deliver approximately 1 liter per day. One section of a Petri dish was embedded with open side downward at one-half of an inch below the surface of the sand in the pot to help distribute the nutrients. To prevent algal growth the reservoirs were covered and all glass which was exposed to the sunlight was painted black. Thirty inches above the bench a framework was constructed to support the capillaries. Compressed air was allowed to bubble through the solution in the supply vessels in order to aerate and stir the culture liquid.

Modifications of Hoagland's nutrient solution were used. In table 1 are given the respective amounts of chemicals (reagent grade) which were diluted with distilled water to make the different culture liquids. Since infection by *Plasmodiophora brassicae* is favored by an acid medium, enough hydrochloric acid was added to keep the sand at about pH 6.5. The small amount of sodium chloride was included in each series in order to eliminate any possible differential effect from the presence or absence of sodium in the various culture solutions.

Table 1.—*Composition of culture solutions used in the study of the effect of host nutrition on clubroot development*

Composition and concentration (moles per liter) of stock solution	Amounts mixed and brought to 18 liters to make up the various solutions					
	Complete	−S	+N	−N	+K	−K
	Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
$Ca(NO_3)_2.4H_2O$, 1.0	9.0	9.0	9.0		9.0	9.0
$CaCl_2.6H_2O$, 1.0				9.0		
KNO_3, 1.0	9.0	9.0	9.0		9.0	
KCl, 1.0				9.0	3.0	
KH_2PO_4, 1.0	1.8	1.8	1.8	1.8	1.8	
NaCl, 1.0	1.0	1.0	1.0	1.0	1.0	1.0
$NaNO_3$, 1.0			4.0	2.0		9.0
$NaH_2PO_4.H_2O$, 1.0						1.8
$MgSO_4.7H_2O$, 1.0	3.6		3.6	3.6	3.6	3.6
$MgCl_2.6H_2O$, 1.0		3.6				
HCl, .1	9.0	9.0	9.0	9.0	9.0	9.0
A-Z [1]	1.8	1.8	1.8	1.8	1.8	1.8

[1] The A-Z stock solution was composed of the following, made up to 1,000 cc. with distilled water: H_3BO_3, 2.818 gm.; $CuCl_2.2H_2O$, 0.040 gm.; $ZnCl_2$, 0.030 gm.; $MnCl_2.4H_2O$, 0.390 gm.; and $FeCl_3.6H_2O$, 5.000 gm.

The proportion of the salts was so arranged as to secure: (1) in the minus-element series, symptoms of deficiency not severe enough to cause death of the plant; (2) in the complete series, moderately growing plants; (3) in the plus-element series, plants showing distinct vegetative stimulation from an extra supply of nitrogen or potassium but no sign of excess symptoms.

Seed was sown in each pot, and after approximately 2 weeks the seedlings were thinned to about 25 plants per pot. To make the inoculum, cabbage clubs were washed and run through a food chopper and, after distilled water was added to the macerate, the suspension was filtered through cheesecloth. The liquid was then centrifuged and when the supernatant liquid had been decanted, more distilled water was added to the residue and the suspension shaken well. The washing process was repeated several times to remove all possible soluble

salts. The resultant gummy spore mass was diluted with enough water, adjusted to pH 6.5 with hydrochloric acid, to provide 100 cc. for each pot. At the first true-leaf stage and 2 weeks later the plants were inoculated with the spore suspension applied to the surface of the sand with a pipette. Plants were pulled and examined for clubroot about 10 weeks after the seed was sown.

Replicate pots were separated by pots of other forms and the arrangement changed each time the experiment was repeated to eliminate variations arising from environmental differences. There seemed to be no appreciable effect from the different positions. All the nitrogen and potassium experiments were run simultaneously in the same greenhouse and one sulfur series was included with these groups; thus the different treatments may be compared to a certain extent. Other trials with sulfur had been made earlier. The composited results for each series represent the totals from two or more experiments unless otherwise designated.

Since McMurtrey (*4*) has described the symptoms on many plants resulting from nutrient deficiency, the appearance of plants grown without sulfur, nitrogen, or potassium will not be described in great detail.

EXPERIMENTAL RESULTS

EFFECT OF SULFUR

Symptoms of sulphur deficiency were quite severe after 4 to 6 weeks. In mustard there was a pronounced difference in pungency between the complete and the minus-sulfur-fed plants when the leaves were tasted. Chemical analysis [4] of one series of sulfur-deficient plants indicated sulfur oils to be practically absent. The sulfur-deficient plants were slightly less green in color and quite stunted. Sometimes a red pigment developed at the leaf margins and near the stem tip. On cabbage there was, in addition to the external symptoms described for mustard, a slight yellowing at the leaf margins and a distinct mottle in the blade. Shogoin and Cowhorn turnips tended to turn more yellow at the leaf margin and show less mottling, while Purple Top Milan turnip was in an intermediate position with regard to symptoms between Shogoin turnip and cabbage.

When the plants were removed and examined for clubbing, three fairly distinct types of diseased roots were noticed. One was the normal, relatively smooth club which occurred most abundantly on the susceptible plants in sulfur-deficient nutrient (fig. 2). Another, found most frequently on resistant plants, was a small gall 1 or 2 mm. in diameter appearing usually, but not always, at the base of a branch root (fig. 3). The third type of overgrowth, seemed to be composed of a great many enlarged galls. These were grouped in the same class as clubs. In some cases these enlargements, which were also more generally prevalent on the resistant strains, became so numerous that they coalesced to form a club very irregular in outline. Figure 4 illustrates this range of symptoms on Cowhorn turnip, a variety intermediate in resistance to clubroot.

In table 2 are the composite results of all the nutrition experiments. The total number of plants and the percentage without visible symp-

[4] The author is indebted to Mark Stahmann of the Department of Biochemistry, University of Wisconsin for making this analysis.

toms, with galls, and with clubs are presented. Sulfur deficiency brought about an increase in the percentage of clubbed plants in both susceptible and resistant[5] strains, the greatest differences being in the resistant hosts. The number of plants with galls in the resistant strains was augmented by the treatment, but, although this type of overgrowth was of rather rare occurrence in the susceptible group, it is to be noted that the trend was always in the direction of more galls in the complete solution. The point is significant, since these en-

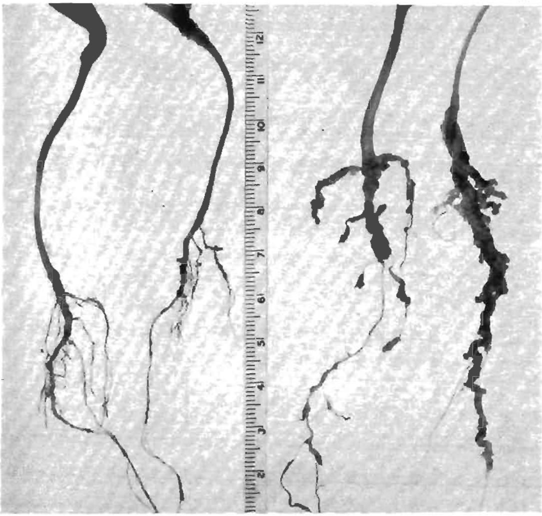

FIGURE 2.—The usual type of club on plants grown in infested sand supplied with artificial nutrient (Shogoin turnip). Scale is in millimeters.

largements were observed most commonly in resistant black mustard in the preliminary trials in soil cited above.

At no time did any evidence of clubroot infection appear on the immune Purple Top Milan turnip.

In one case a few healthy-appearing mustard plants and some mustard plants with galls were thoroughly washed to remove adhering spores of *Plasmodiophora brassicae*. One lot each of healthy and gall-bearing mustard was replanted in sterile sand supplied with a full nutrient solution. A group of each was also set in sand furnished

[5] Unless otherwise designated, the term "resistant" has been used to indicate varieties in which only a few plants show macroscopically visible disease symptoms. Immune strains are ones in which no plants have macroscopic disease symptoms.

with a sulfur-deficient solution. After 1 month there was little change in root symptoms within the group in the complete nutrient solution. In the minus-sulfur set, however, some of the galls had developed into clubs and in one or two cases galls were found on plants which had previously appeared to be healthy. The formation of galls on apparently healthy plants and the development of galls into clubs on diseased plants during this period of deficient nutrition would seem to indicate that some plants in both groups had been infected previous to transplanting. After being transferred to new culture conditions, the plants were evidently able to hold the fungus in abeyance when the nutrition was complete, but when sulfur was

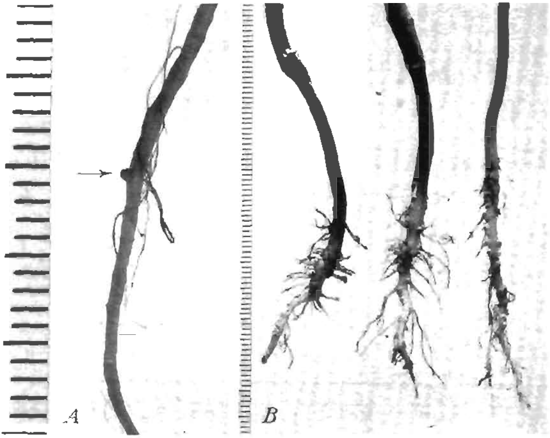

FIGURE 3.—The "gall" type of clubroot symptom on Cowhorn turnip (*A*) and on strain No. 10 of black mustard (*B*). Scale is in millimeters.

absent, more pronounced root symptoms appeared as a result of further invasion by *P. brassicae*. These observations, although made on a small number of plants, seem to conform to those cited above in which the minus-sulfur nutrition tended to increase the percentage of clubs that were normal in size and shape.

EFFECT OF NITROGEN

Three to four weeks after the plants appeared above the surface of the sand, nitrogen-deficiency symptoms became evident. In all varieties the cotyledons turned yellow more quickly on the nitrogen-deficient plants than on those grown with complete nutrients. The leaves were smaller and more yellow in color and a red to purple pigment appeared on the stems usually 2 to 3 weeks before the plants

oped on nitrogen-deficient cabbages than on the plants in the complete nutrient.

In contrast to the sulfur-deficiency symptoms, the characteristic odor and taste of the sulfur oils in the nitrogen-deficient plants ap-

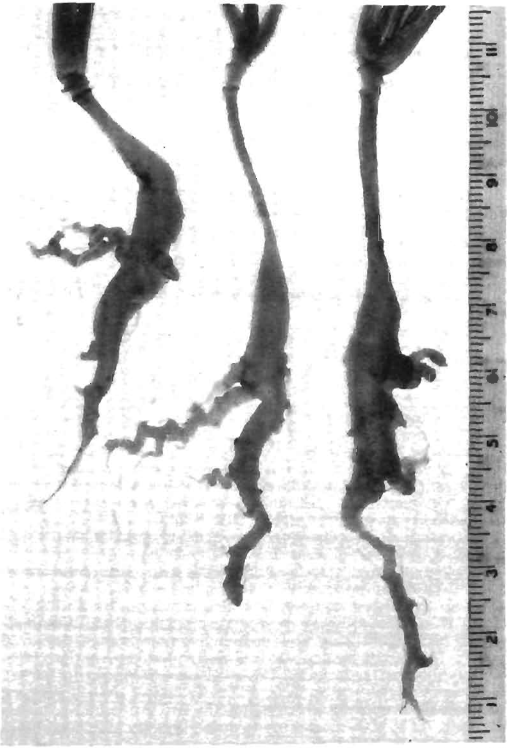

FIGURE 4.—Range of clubbing found on Cowhorn turnip when grown in infested sand supplied with artificial nutrients. Scale is in millimeters.

peared to be just as strong as in the plants in the complete solution. By this crude test there also seemed to be slightly less mustard oil in

TABLE 2.—*The effect of host nutrition on the development of clubroot in certain forms of crucifers*

Variety	Nutrient solution [1]	Total plants	Healthy	Galls [2]	Clubs
		Number	*Percent*	*Percent*	*Percent*
No. 11, black mustard	Complete	330	9.1	1.2	89.7
	−S	130	3.1	.8	96.1
	+N	258	4.6	.4	95.0
	−N	243	1.2	.0	98.8
	+K	71	5.6	.0	94.4
	−K	140	69.3	2.1	28.6
No. 10, black mustard	Complete	333	89.8	3.6	6.6
	−S	219	74.0	10.9	15.1
	+N	236	78.4	9.3	12.3
	−N	223	76.7	7.6	15.7
	+K	74	89.2	2.7	8.1
	−K	159	93.7	1.9	4.4
Shogoin turnip	Complete	344	26.2	2.3	71.5
	−S	158	6.9	1.3	91.8
	+N	192	10.4	2.1	87.5
	−N	181	13.8	2.2	87.5
	+K	69	14.5	.0	85.5
	−K	215	82.3	.0	17.7
Cowhorn turnip	Complete	204	63.7	5.4	30.9
	−S [3]	72	30.6	11.1	58.3
	+N	227	52.9	14.1	33.0
	−N	224	51.3	4.5	44.2
	+K [3]	90	81.1	.0	18.9
	−K	217	96.3	.5	3.2
Purple Top Milan turnip	Complete	125	100.0	.0	.0
	−S	112	100.0	.0	.0
	+N	34	100.0	.0	.0
	−N	29	100.0	.0	.0
	+K	51	100.0	.0	.0
	−K	50	100.0	.0	.0
Jersey Queen cabbage	Complete	328	17.4	1.2	81.4
	−S	137	8.0	.0	92.0
	+N	193	11.4	.5	88.1
	−N	207	7.7	.0	92.3
	+K	62	6.5	1.6	91.9
	−K	190	77.9	2.6	19.5

[1] See table 1. [2] See figure 3. [3] 1 experiment only.

The nitrogen-deficient plants were much smaller than the plants in any other series, yet clubs formed vigorously, most of the few branch roots which appeared as well as the main tap root being entirely hypertrophied. Nitrogen starvation was very similar to sulfur deficiency in its effect on clubroot development (table 2). The symptoms were of the same type (figs. 2, 3, 4) and the percentage of plants with clubs was increased markedly as compared to those in the complete solution. Susceptible plants with galls were fewer in number than those grown with the complete solution; however, gall formation on resistant varieties was more erratic. In No. 10 black mustard there was a larger proportion of plants with galls and in Cowhorn turnip galls were fewer than when these two varieties were furnished the complete nutrient solution. No explanation of this observation seems forthcoming from the present data.

The plus-nitrogen plants were distinctly larger than those grown in the complete nutrient solution and were of a somewhat deeper green color. The clubs in this group were also very large as compared to those produced in the minus-nitrogen and complete-nutrient series. More nitrogen than that furnished in the complete solution aided clubroot development. The increase in clubbing of high-nitrogen plants over those grown with complete nutrients was less in resistant than in the susceptible strains. Where there was increased suc-

culence and rate of growth in the host, induced by an extra supply of nitrogen, a larger percentage of clubs developed than in the complete solution. The positive correlation of vigorous growth of the host and rapid development of the parasite has been observed commonly with other diseases. With respect to the appearance of galls, high nitrogen tended to have the same influence as low sulfur. The proportion of plants with galls in susceptible forms was less than that in the complete solution, while the opposite was true for the resistant strains.

As in the sulfur series, Purple Top Milan turnip proved to be immune regardless of the variation in nitrogen supply.

EFFECT OF POTASSIUM

The first symptom of potassium starvation appeared as a definite yellowing of the foliage 5 to 7 weeks after the cotyledons broke through the sand. The plants were somewhat smaller than those grown with complete nutrients but the reduction in size was not nearly as great as that caused by deficiencies of the other two elements. In 6 to 8 weeks severe necrosis appeared on these lower leaves both at the margin and in spots on the blade. These lower leaves later became dry but did not drop, and gradually the necrosis appeared on leaves higher on the stem. No difference in mustard-oil content could be detected between the plants at any of the potassium levels.

The same types of clubroot symptoms appeared on these plants as on those grown in the other nutrients already described. The largest change in amount of infection was brought about by potassium deficiency (table 2). The reduction in disease development as a result of this deficiency was approximately 60 percent in the susceptible varieties when compared with the percentage of infection of these same varieties furnished a complete supply of nutrients. The amount of reduction seemed to depend on the degree of resistance of the strains used, the greater effect being with the susceptible lines. With No. 10 black mustard where complete-nutrient plants developed 6.6 percent clubs, the percentage was reduced by only 2.2 percent; while with Cowhorn turnip, which was 30.9 percent clubbed in the complete solution, the percentage was reduced by 27.7 percent. The appearance of galls on the susceptible varieties showed no constant variation, but on the resistant strains the percentage of plants with galls tended to decrease with an insufficiency of potassium.

In the plus-potassium series the plants were somewhat larger than those grown in the complete solution and slightly smaller than those in the plus-nitrogen series. With all the varieties except Cowhorn the proportion of plants with clubs was augmented and on all varieties the appearance of galls was decreased as compared with plants in the complete solution.

It should be pointed out that no clubroot symptoms were found on Purple Top Milan turnip in the potassium series.

DISCUSSION

Although it has been reported difficult to secure clubroot on plants in sand culture (*5*) with the methods reported herein, crucifers in artificial nutrient solution were almost as severely attacked as were those grown in heavily infested soil. With some forms the percentage of

infection was higher and with others lower than when plants of the same variety were grown in infested soil. No explanation for this situation seems adequate at the present time.

Corroborating the work of Gassner and Hassebrauk (*3*), the results of these tests indicated that varying nutrition had the most pronounced effect on disease development in hosts which were to a certain degree susceptible, but did not influence resistance in plants such as Purple Top Milan, a variety of turnip immune to Wisconsin collections of the pathogen. Resistance does not seem to be correlated with vegetative vigor. There was a wide difference in the percentage of clubs in the N-, S-, and K-deficient series even though all of the plants showed reduced vigor. These observations are similar to those made on other diseases (*13*, *15*). In contrast to these other cases, however, it was noticed that the enhanced growth which resulted from an extra supply of nitrogen or potassium also promoted the development of clubs, although not as pronouncedly as did the deficiency in sulfur or nitrogen.

Since *Plasmodiophora brassicae* has not been grown in pure culture it is not easy to determine whether the elements used affect the parasite directly. Sulfur and nitrogen at least do not seem to be necessary in abundance for the parasite to act as a virulent pathogen, while the absence of potassium either decreases penetration or affects the host in such a way as to be unfavorable to *P. brassicae*. In relation to this latter element, it is of interest to note the analysis of clubbed and healthy cabbage roots made by Reed (*8*). He found that although the ash constituents were present in appreciably greater amounts in diseased than in healthy tissue, the greatest increase of any single element was in the case of potassium. In view of this analysis and the fact that potassium deficiency markedly decreased the number of clubs, it may be that the element is necessary for the growth processes of the parasite as well as those of the host.

Since decreasing the amount of nitrogen without appreciably lowering the mustard-oil content increased clubroot infection to about the same degree as eliminating sulfur in the culture solution, sulfur oils do not appear to be necessary to the host in preventing or retarding development of *Plasmodiophora brassicae*. Upon the basis of the data obtained in the present investigation it is not possible to say why pronounced nitrogen and potassium deficiency should not greatly lower the amount of sulfur oil.

Plasmodiophora brassicae subsists in living host cells either directly upon the host protoplasm or upon products produced or absorbed by the host cell. In either case its existence in the vegetative stage is dependent upon the living plant. As a result any marked disturbance of host metabolism by mineral deficiency might affect the relationship with the parasite in such a way as to increase the competition for elaborated food and perhaps for the mineral nutrients. Sulfur, nitrogen, and potassium being essential elements, are necessary not only because they are constituents of certain indispensable compounds, but also because they influence the formation of requisite substances in which they are not present—notably the carbohydrates. Thus decreased resistance may be due to the absence of certain compounds, to the accumulation of others, or to metabolic processes which are as yet unknown. Immunity such as that occurring in Purple Top

Milan turnip must be even more closely related to the host protoplasm since drastic changes in host metabolism did not alter the ability of the plant to prevent clubroot development in its tissue.

SUMMARY

Methods have been reported whereby clubroot infection was secured in sand culture. The percentage of diseased plants was comparable to that obtained in infested, acid soil. Upon the basis of symptoms, plants were grouped into three classes: (1) Those with no symptoms, (2) those with clubs, (3) those with galls. The clubs were of the usual type mentioned by other workers. The galls were small spherical overgrowths, 1 or 2 mm. in diameter, appearing usually, but not always, at the base of a branch root and being more prevalent on the resistant varieties.

The effect of sulfur, nitrogen, and potassium nutrition on the development of clubroot in susceptible, resistant, and immune strains of crucifers was studied. The proportion of nutrients in the different experiments was adjusted so as to produce: (1) Plants showing deficiency symptoms, (2) plants growing moderately, and (3) plants having pronounced vegetative vigor resulting from an extra supply of nitrogen and potassium.

The percentage of susceptible plants having clubs was in general increased slightly over that in the complete solution by an abundance of potassium, more by an abundance of nitrogen, and most by the absence of sulfur or nitrogen. The percentage was decreased markedly in plants deficient in potassium. A smaller proportion of plants of susceptible strains developed galls when grown in nutrient solutions in which sulfur or nitrogen was withheld than was the case with resistant strains.

The percentage of resistant plants having clubs was increased somewhat by high nitrogen, but it was greatest in the cases of sulfur or nitrogen deficiency. With an extra amount of potassium the results were inconclusive. The proportion of plants with clubs was decreased definitely by lack of potassium. The formation of galls was usually increased by the absence of sulfur or by high nitrogen and was generally decreased by high or low potassium, but the effect of the other nutrition series on the presence of these overgrowths was not conclusive.

No signs of clubroot appeared regardless of the variation in nutrient supply on the immune variety of turnip, Purple Top Milan.

Deficiency of sulfur lowered the sulfur-oil content greatly in the crucifer varieties while nitrogen starvation did not. On a given variety, the number of plants with clubs was increased to approximately the same extent by the absence of either of these elements. From these observations it would appear that the sulfur oils are not essential in enabling the host to prevent or retard clubroot development in its tissues.

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EFFECT OF CORN, WHEAT, AND BARLEY IN THE DIET ON THE PHYSICAL AND CHEMICAL COMPOSITION OF FRYERS AND ROASTERS [1]

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INTRODUCTION

While much has been learned from studies of the effects of grains on growth and egg production in poultry, somewhat less is known of the effects of grains upon the physical and chemical composition of the meat produced. In 1936, the writers began a series of experiments which involved a comprehensive study of the effects of simple rations composed principally of corn, wheat, or barley upon the physical and chemical composition of fryers and roasters. The tests were made with five general objectives in mind, namely, to determine: (1) The rate of gain in body weight of birds on different feeds and the amount of feed required to produce a unit of gain; (2) the association of body weight and observed grade with certain external measurements of the dressed carcass; (3) the relationship of these body measurements to the total amount of edible meat; (4) the effect of the different cereals upon the quantity of light meat, dark meat, skin and subcutaneous fat, and abdominal fat; and (5) the effects of the cereals upon the deposition of fat in these different classes of edible meat as an indication of edible quality.

REVIEW OF LITERATURE

Cruickshank (*3*) [2] reported that the fat from birds fed barley was firmer than that from birds fed corn; however, the fat of the corn-fed birds was not objectionally soft. It was also noted by Gutteridge (*4*) that the feeding of White Leghorn capons in pens was relatively inefficient as compared to the feeding in fattening crates.

With reference to the effect of environment on fattening poultry, MacDonald and McMurray (*13*) stated that during the summer months trough-fed range birds made as good gains as trough-fed birds kept in a fattening shed. With roasters, it was found by Harshaw (*5*) that the percentage of protein and ash was significantly higher in the leg muscle of range birds, and that the protein content of the breast muscle was also higher. The percentage of fat in the edible portions was generally higher in the range-reared than in the confined birds, but the differences were not statistically significant.

Harshaw (*6*) reported that the absolute gain in live weight during fattening increased with age, but the relative gain decreased. The ratio of the leg muscle to dressed weight increased with age, but the

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[2] Italic numbers in parentheses refer to Literature Cited, p. 177.

ratio of the breast muscle to dressed weight remained practically the same. The ratio of the remaining edible portion to dressed weight depended largely on the extent of fattening. In younger birds the moisture increased more than the fat during fattening, but in older birds the reverse was true. The percentage of breast muscle and leg muscle decreased with fattening, while the percentage of the remaining edible portion increased. The fat increased in all edible portions during fattening, but the greatest quantity of new fat was laid down in the "remaining" edible portion (comprising all the edible portions except the breast and leg muscle). Fattening resulted in a decrease in the percentage of protein, ash, and water in the remaining edible portion and the leg muscle, but not in the breast muscle.

Harshaw (*5*) found that the percentages of breast muscle, leg muscle, and the total edible portion were significantly higher for range-reared birds than for confined birds. The percentage of fat was higher in all the edible portions of the fattened birds as compared with that in the unfattened birds. The fattened birds had an average of 85 percent more fat in the breast muscle, 43 percent in the leg muscle, and 57 percent in the remaining edible portion as compared with the unfattened birds. There was usually a lower percentage of protein, ash, and water in the edible portions of the fattened birds, although there was actually an increase in the total amounts of protein, ash, and water. The leg muscle contained a larger proportion of water than the other edible portions.

In a study by Maw et al. (*18*) of Barred Plymouth Rock roasters, it was found that the fat in the edible meat of corn-, wheat-, oat-, and barley-fed birds amounted to 8.95, 6.99, 4.81, and 3.43 percent, respectively. It was further found by Maw and Maw (*21*) that corn meal caused a high percentage of the total body fat to be deposited in the flesh and much less to be deposited in the abdominal cavity and the skin of the bird; whereas the cereals barley, oats, and wheat showed the reverse in varying degrees. On the other hand, Gutteridge (*4*) reported a significant correlation between the percentage of fat in the skin and subcutaneous tissue and that in the abdomen, indicating that fat was distributed in a definite and similar ratio in the depot areas, regardless of the feed given. Crampton (*2*) has presented a good review of the comparative feeding values of the cereals.

More recently, Maw (*17*) noted that single cereal grains gave as good results as combinations of cereals. Corn was superior to wheat, oats, and barley in producing edible meat and fat as well as skin and abdominal fatty tissue. In this work (*17*) the character of body fat was also studied as to iodine number and color.

Maw et al. (*22*) reported that mature roasters fed corn had the largest amount of total fat, those fed wheat the next largest, and those fed barley and oats had least. The length of the fattening period affected the interpretation of the values of these cereals. Corn tended to produce more fat in the flesh and less fat in the skin and abdominal regions, whereas wheat produced more skin and abdominal fats and less flesh fats. In another report, Maw and Maw (*21*) stated that the rations fed had no significant effect upon the distribution of fat in the edible portion of Leghorn broilers.

Physical and chemical studies were made by Harshaw (*7*) on five lots of 12-week-old cockerels. One lot was fed a commonly used

control all-mash ration and the other four were fed rations composed each of corn, wheat, oats, or barley as the only cereal grain with protein, vitamin, and mineral supplements. As compared with the control, the oat- and barley-fed lots had about the same proportion of leg and breast muscle; the wheat- and corn-fed lots had considerably less. The percentage of the remaining edible portion was approximately the same for all five lots. Considering the percentage of total edible portion, the order of the five lots was as follows: Control birds, oat-, barley-, wheat,- and corn-fed. The corn-fed birds were the highest in fat content of edible portions, followed in order by those fed the wheat, control, barley, and oat diets. No difference in the distribution of fat between the edible portions was noted in the birds on the different diets.

A method has been reported by Lloyd (*12*) for estimating the amount of breast fleshing according to which classifications are made of the so-called U, V, and I breasts. The method would serve as a guide in selecting breeding stock for egg production.

Body measurements have been taken by several investigators. Jaap and Penquite (*10*) noted that differences in body conformation of live and dressed birds may be accurately expressed by comparing body weight, shank length, keel length, and minimum anterior body depth. They found that a satisfactory point for measuring breast width is about 1½ inches from the anterior end of the keel on a line toward the insertion of the femurs. Jaap (*9*) also reported breed differences in body conformation as compared with shank length. For example, Cornish and Game breeds attain a broad, plump breast at an early age, whereas American and English class breeds reach their best market shape at maturity. Brahmas and Giants belong to a group that is thin and angular during growth and only at maturity attains superior body shape. Lerner (*11*) concluded that shank length measured on live birds forms a valid criterion in studies on inherited size differences in fowls. Lloyd (*12*) reported no correlation between meat type and egg production. Market type, however, was judged without the aid of definite body measurements. Maw (*15*) and Maw and Maw (*14, 20*) have reported on body measurements; they (*14*) found that correlations existed between fattening gains and certain body measurements, which included body length, leg length, and circumference of tibial muscles.

EXPERIMENTAL METHODS

PROCEDURE IN 1936

The first experiment was started in the spring of 1936 with White Plymouth Rock cockerels, which were studied as roasters. During the first 8 weeks the chicks were kept in electrically heated starting batteries. The all-mash starting rations used included 46 pounds of a basal mixture made up of 10 percent of meat and bone scraps used for the first 4 weeks; 15 percent of meat scraps from the fourth to the eighth week; 10 percent of dried buttermilk the first 4 weeks and 5 percent of dried milk from the fourth to the eighth week; pulverized oats 20 percent, alfalfa leaf meal 5 percent, and cod-liver oil 1 percent. To this mixture was added 54 pounds of either ground yellow corn, wheat, or barley. Ninety day-old chicks of both sexes [illegible] but only the male survivors

were continued on the growing rations. Each of the three groups received the same principal grain that it had received during the starting period. These rations were given throughout the entire growing period (8 to 31 weeks). No finishing rations were tested in 1936. The cockerels were kept in colony brooder houses with access to millet and rape range.

On November 10, at 31 weeks of age, the 10 cockerels whose weights were nearest the mean weight for each group were selected and dressed (semiscald method) after having been starved for 24 hours. They were then chilled at 36° F., weighed, graded, and the body measurements taken. Then they were dissected and the different classes of edible meat separated for chemical analysis. Only one-half of the carcass was used for this analysis.

PROCEDURE IN 1937

Barred Plymouth Rocks were used in the second experiment, which was started on May 5, 1937. About 70 cockerels 8½ weeks of age and of practically equal average weight were placed in each of three colony brooder houses. They were given growing rations similar to those used in 1936 and had access to millet and rape green range.

Finishing rations for fryers and roasters were studied both in batteries and on the range. With fryers, the 3-week finishing period began when the birds were 14 weeks of age and ended at 17 weeks; with roasters, the finishing period began at 24½ weeks and ended at 26½ weeks. With both fryers and roasters, only those 17 birds from each lot whose weights were nearest the average for the lot were selected for battery and range studies of finishing rations. The standard error for body weight was thus reduced. Although the number of individuals studied was also reduced it is believed that these birds were representative of the lots from which they were taken.

At the end of the 2- or 3-week finishing period, 10 birds whose average weights were nearest the average for the lot were selected from each 17-bird group finished on the range and in the battery. These birds were dressed, graded, body measurements were taken, and each carcass was cut in half. One half was used for chemical analyses and the other half for flavor studies (*23*). The methods of analysis were the same as those employed in the 1936 experiments.

DETERMINING DRESSED GRADES

Grades were determined by placing all the carcasses from all the different lots together and aligning them according to the order of breast fleshing. On the basis of breast fleshing, it was then estimated that the best 25 percent of all the dressed carcasses would grade U. S. Special, the next 25 percent U. S. Prime, the third 25 percent U. S. Choice, and the last 25 percent U. S. Commercial. In order to determine the average grade of each group, numerical values of 1, 2, 3, and 4 were given to each U. S. grade. The band numbers of all birds in the different grade classifications were then determined and grouped according to the rations used and the average numerical grade values calculated. By this method, the grader did not know what rations the birds had received until after the grades for all the birds had been determined.

BODY MEASUREMENTS AND GRADES

The present methods of determining the U. S. grade of live or dressed poultry are based upon the observations and opinions of the official grader, and often there is much difference of opinion among graders as to how a bird should be classified. This fact has decided economic importance since lower grades are penalized considerably on the market.

Since the amount of flesh and subcutaneous fat on the carcass has an important influence upon the observed grade, several body measurements were taken with a view to selecting those measurements that are most closely associated with the actual amounts of edible flesh and fat present.

Figure 1, *A*, shows the breast width measurement which was taken five-eighths inch back from the anterior tip of the sternum. A second

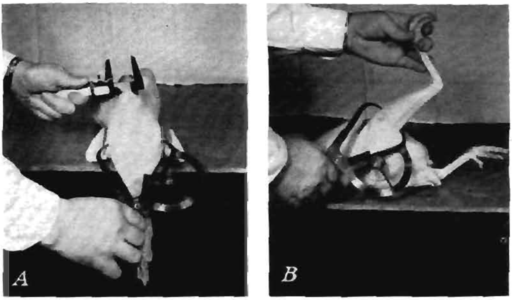

FIGURE 1.—*A*, Breast-width measurement taken five-eighths of an inch from the anterior tip of the sternum with a vernier caliper and shoulder width taken at the widest point with an indicating caliper; *B*, anterior-posterior tibia measurement.

measurement was taken 3 inches back from the anterior tip of the sternum. Both of these measurements, taken with a vernier caliper, give an indication of the breast angle and the amount of flesh at the edge of the sternum. Figure 1, *A*, also shows the location of the shoulder width measurement taken at the widest point. A third measurement (fig. 2, *A*) was taken with an indicating caliper 3 inches back from the anterior tip of the sternum and 1½ inches from the edge at the anterior end of the lateral feather tract. The breast length was simply a measurement of the length of the sternum from anterior to posterior tips. Figure 2, *B*, shows the method of determining the hip width at the widest part of the ilium. The femur and tibia measurements were taken to represent the length of these two bones at the joints when the tibia and femur were held at right angles. Figure 1, *B*, shows the method of taking anterior-posterior tibia measurements at the widest point by holding the metatarsus at a right angle to the tibia. By holding the instrument at right angles to the position of this anterior-posterior measurement, the distance across

the tibia flesh was determined at the thickest point. No femur or tibia measurements were taken for either the roasters reared in 1936 or the fryers in 1937.

INDEX VALUE

Width of breast ⅝ of an inch and 3 inches from the anterior tip of the sternum were the two measurements of greatest value in determining the amount of flesh present. By considering also the length

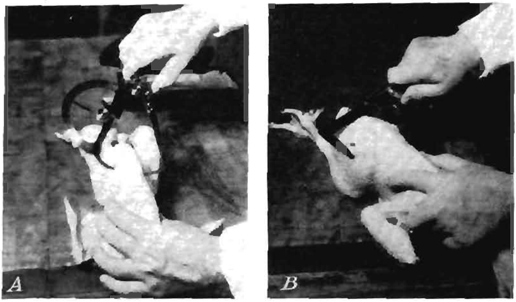

FIGURE 2.—*A*, Breast width measurement taken with an indicating caliper 3 inches from the anterior tip of the sternum and 1½ inches from the edge of the anterior end of the lateral feather tract; *B*, measurement of the hip width taken with a vernier caliper.

of breast and combining the three measurements, an index value was found for each bird according to the equation

$$I = l\,(A^2 + B^2)$$

where

I = index value.
l = breast length.
A = width of breast ⅝ of an inch from tip.
B = width of breast 3 inches from tip.

These measurements are most suitable for roasters. The index number obtained by the foregoing equation places most value on breast measurements and has the advantage of permitting statistical treatment.

SEPARATION OF EDIBLE MEATS

The neck was removed and each carcass was cut longitudinally through the spinal column and breastbone, so that there was about the same amount of edible meat on each half of the carcass. All the edible meats were quickly removed from one-half of each carcass, then weighed and classified as to (1) light meat, including the breast and wing muscles; (2) dark meat, including the muscles over the femur, tibia, and ilium; (3) skin and subcutaneous fat, including the fat which adhered to the skin as the skin was pulled from the muscles; and (4) abdominal fatty tissue, which included all fat deposited in the abdomi-

nal cavity. This did not include the fat adhering to the digestive tract. Each of the four samples from each carcass was placed in an airtight sample bottle and stored in a refrigerator at about 35° F. for chemical analysis.

CHEMICAL ANALYSIS OF MEAT

At the time of analysis, the samples were ground and thoroughly mixed by running them through a small meat grinder 10 times. Moisture and fat determinations were both made on the same sample. The samples were introduced (about 3 gm.) into weighed 18-gm. 8-percent butterfat test bottles by a specially made heavy metal syringe which forced the meat out in a filament about 2 mm. in diameter. The bottles were rotated rapidly as the samples were introduced, thus depositing the filament of meat around the outer edges of the bottles and exposing a large surface for rapid loss of moisture. The bottles were placed in a vacuum oven at 28° C. for 36 hours at a pressure of not over 2 mm. of mercury. Under these conditions, very little difficulty was experienced with volatilization of fat.

The fat was determined volumetrically on the dried samples in the butterfat test bottles by a technique developed to eliminate the errors due to the volatility of the chicken fat. Allen (*1*) has recently published a volumetric method for the determination of blood fat. Ten cubic centimeters of 10-percent ammonium hydroxide was added to soften the dried filaments of meat, and 10 cc. of 50-percent sulfuric acid was then introduced to digest the proteins. Warm water was added and the bottles were centrifuged until a clear separation of the fat was obtained in the neck of the test bottles. Readings were made at 98° C. and corrected for the specific gravity of the fat at that temperature.

STATISTICAL ANALYSIS OF DATA

The data have been treated statistically by the analysis of variance method as discussed by Snedecor (*24*). In analyzing the variance in tables with disproportionate subclasses, the method of expected subclass numbers has been used. In all calculations it was assumed that the data represented a random sampling of a normal distribution.

Standard errors of all quantities were determined, and significant differences between means of quantities were determined for the probability of 19 to 1, and for highly significant differences for the probability of 99 to 1. In each table, all differences between means have been tested for significance. Differences which are not significant (odds less than 19 to 1) have not been indicated in the tables. The words "slightly" and "somewhat" are frequently used in the text to denote a trend (odds 19 to 1), but in no case have definite conclusions been based upon trends. The word "appreciable" as used in this report is synonymous with "highly significant."

Tests for significant differences in most cases were made by the usual t test (Snedecor, *24*), in which the standard error of the difference was taken to be equal to the square root of the sum of squares of the standard errors of the means. In several border-line cases (perhaps due to unequal frequencies, but more often to rounded-off numbers) significant differences might have been expected where none are shown. This was due to the fact that the more exact statistical treatment sometimes eliminates the border-line individuals.

EXPERIMENTAL RESULTS

The data presented in table 1 show the growth of the birds, the rations fed, and the feed consumed in the experiments conducted in 1936 and 1937. It is apparent from table 1 that the cockerels that received the barley ration did not gain as much in weight as those that received the wheat or corn in 1936, and there were no highly significant differences between the gains in body weight on any of the three grains in 1937. With respect to the amount of feed required to produce a unit of gain in body weight for roasters, corn was only slightly superior to wheat, while considerably more of the barley ration was required. This agrees with the report of Maw and Maw (*21*), who found the gain-feed ratio in favor of corn, followed in order by wheat and barley. For the fryers produced, wheat appeared most efficient, barley less so, and corn least.

TABLE 1.—*Average weights during growing period as related to feed consumption, and relative values of corn, wheat, and barley rations when fed to range-reared White Plymouth Rock roasters in 1936 and to Barred Plymouth Rock fryers and roasters in 1937*

Ration	Birds at beginning of test	Age of birds at beginning of test	Average initial weight	Birds at end of growing period	Age of birds at end of growing period	Average weight at end of growing period	Gain in weight [2]	Feed per unit of gain	Feed consumed per bird	Grain as proportion of total feed intake	Grain in total ration actually consumed during growing period
	No.	*Weeks*	*Grams*	*No.*	*Weeks*	*Grams*	*Grams*	*Grams*	*Lb.*	*Pct.*	*Pct.*
WHITE PLYMOUTH ROCK ROASTERS, 1936 [1]											
Corn	38	8	[4] 449.2	28	31	2,946.3	2,548.9	8.84	49.64	41.5	78.9
Wheat	57	8	[4] 543.6	33	31	[4] 3,055.1	[5] 2,520.4	8.86	49.21	55.1	83.8
Barley	56	8	507.4	44	31	2,799.2	2,296.3	9.11	46.08	48.2	81.4
BARRED PLYMOUTH ROCK FRYERS, 1937 [6]											
Corn	70	8½	549.5	69	14	[7] 1,135.6	[7] 586.1	6.08	7.85	32.5	72.3
Wheat	70	8½	554.3	70	14	1,227.0	672.7	5.07	7.52	32.7	72.4
Barley	72	8½	556.3	68	14	1,164.4	608.1	5.56	7.45	31.5	71.0
BARRED PLYMOUTH ROCK ROASTERS, 1937 [6]											
Corn	44	16½	1,568.6	42	24½	2,252.2	682.4	7.17	10.77	58.4	82.9
Wheat	45	16½	1,636.0	44	24½	2,290.8	648.9	7.39	10.56	73.2	89.0
Barley	46	16½	1,594.9	46	24½	2,239.7	644.8	8.91	12.65	60.1	83.6

[1] 64 percent of grain was added to the following basal mixture: Pulverized oats 20 percent, meat and bone scraps 10, dried buttermilk 5, and salt 1 percent. The same principal whole grain used in the mash was also given to each lot ad libitum as their sole grain.
[2] Gains were calculated by subtracting the initial weights of survivors from their final weights. In the corn group, for example, the average initial weight of the survivors was 430.9 gm. Initial weights given include the average weights of all cockerels started on the experiment.
[3] Highly significant greater response to wheat and barley than to corn.
[4] Significantly greater response to wheat than to barley.
[5] Highly significant greater response to wheat than to barley.
[6] 59 percent of grain was added to the following basal mixture: Pulverized oats 20, meat and bone scraps 10, alfalfa-leaf meal 5, dried buttermilk 5, and salt 1 percent. The same principal whole grain used in the mash was also given to each lot ad libitum as their sole grain.
[7] Significantly greater response to wheat than to corn.

The percentage of grain of the total feed consumed varied considerably with the roasters. This was due to the fact that both the growing ration of mash and the grain were fed ad libitum in hoppers and there was a tendency for the cockerels receiving either wheat or barley to consume more of these grains in proportion to mash than those receiving

corn. Consequently, a somewhat larger percentage of the total ration ingested consisted of wheat, followed in order by barley and corn. There were practically no differences between the fryer groups, as will be noted in the last two columns of table 1.

During the finishing period studied in the 1937 experiments, an all-mash ration was used. Consequently, the percentage of cereal grain in the total ration consumed remained constant at 62 percent.

TABLE 2.—*Average weights of birds during finishing period as related to feed consumption, and relative value of corn, wheat, and barley when fryers and roasters were range- and battery-fed in 1937* [1]

Ration	Birds finishing test	Age	Average initial weight	Birds at end of finishing period	Age of birds at end of finishing period	Average weight at end of finishing period [2]	Gain in weight [3]	Feed per unit of gain	Feed consumed per bird
	Number	*Wks.*	*Grams*	*Number*	*Weeks*	*Grams*	*Grams*	*Grams*	*Pounds*
RANGE-FED FRYERS									
Corn	16	14	1,156.7	15	17	[4] 1,685.5	[4] 519.5	3.87	4.43
Wheat	16	14	[5] 1,219.0	16	17	[6] 1,682.0	[6] 463.0	4.20	4.20
Barley	17	14	1,175.1	17	17	1,547.1	372.0	5.46	4.48
BATTERY-FED FRYERS									
Corn	17	14	1,149.6	17	17	1,571.8	422.1	3.70	3.44
Wheat	16	14	[5] 1,217.3	16	17	1,604.5	387.2	4.31	3.68
Barley	17	14	1,173.6	17	17	1,633.2	[7] 459.6	4.18	4.24
RANGE- AND BATTERY-FED FRYERS COMBINED									
Corn	33	14	1,153.1	32	17	1,625.1	[8] 467.8	----	
Wheat	32	14	[9] 1,218.2	32	17	1,643.3	425.1	-	-
Barley	34	14	[10] 1,174.3	34	17	1,590.1	415.8	----	--
RANGE-FED ROASTERS									
Corn	17	24½	2,291.1	17	26½	2,532.8	241.6	6.57	3.50
Wheat	17	24½	2,294.0	17	26½	2,515.4	231.1	6.98	3.56
Barley	16	24½	2,223.3	16	26½	2,416.4	193.1	9.36	3.98
BATTERY-FED ROASTERS									
Corn	17	24½	2,284.2	16	26½	[4] 2,660.6	[4] 380.8	5.62	4.72
Wheat	17	24½	2,283.7	17	26½	[11] 2,583.8	[6] 300.1	7.16	4.73
Barley	17	24½	2,246.5	17	26½	[11] 2,443.0	210.2	10.16	4.71
RANGE- AND BATTERY-FED ROASTERS COMBINED									
Corn	34	24½	2,287.7	33	26½	[4] 2,594.8	[4] 309.1	----	----
Wheat	34	24½	2,288.8	33	26½	[11] 2,550.6	[6] 266.6	----	----
Barley	33	24½	2,235.2	33	26½	2,430.1	201.7	----	----

[1] *62 percent of grain was added to the following basal mixture: Pulverized oats 20 percent, dried butter*milk 13, meat and bone scraps 5. Range-fed birds received the dry mash; battery-fed birds received the mash with water mixed in to make a paste of the proper consistency, the corn mixture requiring 55, the wheat 58, and the barley 62 percent of moisture for the fryers, and 60, 62, and 65 percent for the roasters.

[2] The response of corn-fed fryers was significantly greater on the range than in the battery.

[3] *The response of both corn-fed and wheat-fed fryers was highly significantly greater on the range than in* the battery; the response of both barley-fed fryers and corn-fed roasters was highly significantly greater in the battery than on the range; the response of wheat-fed roasters was significantly greater in the battery than on the range.

[4] Highly significantly greater response to corn than to barley.

[5] Significantly greater response to wheat than to corn.

[6] Highly significantly greater response to wheat than to barley.

[7] Highly significantly greater response to barley than to wheat.

[8] Significantly greater response to corn than to barley.

[9] Highly significantly greater response to wheat than to corn.

[10] Significantly greater response to wheat than to barley.

[11] Significantly greater response to wheat than to barley.

Table 2 gives the average body weights and gains during the finishing period for the fryers and roasters produced in 1937. No finishing

tests were conducted in 1936. Contrary to the results of 1936, no appreciable differences were found in the average rates of gain in body weight between the corn-, wheat-, and barley-fed fryers and roasters. During the finishing period of 1937, the corn- and wheat-fed groups usually gained somewhat more than the barley-fed groups. In comparing the birds finished on the range with those finished in the battery, it is evident that the range-reared fryers receiving either corn or wheat gained appreciably more than the fryers finished in batteries. The reverse was true with those receiving barley. With the roasters, however, both the corn- and wheat-fed lots finished in batteries gained appreciably more than the corresponding lots finished on the range. There were no appreciable differences between the range and battery groups receiving barley, nor were there any appreciable differences in the amount of feed required to produce a unit of gain between the grains used during the finishing period.

From table 3, which gives dressed weights and grades, it is evident that the corn- and wheat-fed lots were heavier than the barley-fed lot in both years; there were no appreciable differences between the corn- and wheat-fed lots. The fryers receiving wheat on the range were appreciably heavier than those receiving wheat but confined to batteries for the 3-week finishing period. Of the roasters finished in 1937, those fed corn in the battery were heavier than those fed corn on the range. Other differences between battery- and range-finished birds were not significant. Although there appeared to be differences in the dressed grades, there was so much variation among the individuals receiving the same ration and treatment that these differences were not statistically significant.

TABLE 3.—*Average dressed weights, dressing percentages, and grades of dressed carcasses of fryers and roasters fed corn, wheat, and barley on range or in battery, 1936–37*

Type of bird, year, and ration	Average dressed weights (10 birds in each lot)			Ratio of drawn weights to live weights			Mean grade of carcasses for range and battery combined
	Range	Battery [1]	Range and battery combined	Range	Battery [2]	Range and battery combined	
	Grams	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	
Fryers (1937):							
Corn	[3] 1,162	1,140	[3] 1,151	[3] 71.1	[3] 70.9	[3] 71.0	1.8
Wheat	[4] 1,197	1,116	[4] 1,156	[4] 71.0	69.4	[4] 71.9	1.3
Barley	1,049	1,098	1,074	68.0	67.9	67.9	1.6
Roasters (1937):							
Corn	1,862	[3] 2,021	[4] 1,942	72.1	[5] 75.4	73.8	[5] 1.6
Wheat	1,865	[6] 1,840	[7] 1,852	73.4	[6] 71.2	72.3	1.9
Barley	1,798	[8] 1,726	[8] 1,762	72.2	71.9	72.0	2.0
Roasters (1936):							
Corn	[3] 2,094			[5] 68.2			[9] 2.4
Wheat	[10] 2,195			[8] 69.2			[9] 1.8
Barley	[4] 1,907			65.2			[9] 2.4

[1] The response of wheat-fed fryers was highly significantly greater on the range than in the battery; the response of barley-fed fryers was significantly greater in the battery than on the range; the response of corn-fed roasters (1937) was significantly greater in the battery than on the range.

[2] The response of the corn-fed roasters (1937) was highly significantly greater in the battery than on the range.

[3] Highly significantly greater response to corn than to barley.

[4] Highly significantly greater response to wheat than to barley.

[5] Significantly greater response to corn than to barley.

[6] Highly significantly greater response to corn than to wheat.

[7] Significantly greater response to corn than to wheat.

[8] Significantly greater response to wheat than to barley.

[9] Range-fed birds only.

[10] Significantly greater response to wheat than to corn

Table 4 gives the distribution of fleshing in fryers and roasters. With the fryers, there was a higher percentage of light meat in the total edible meat of the wheat-fed birds than in that of the corn-fed birds. Similar differences were noted by Maw and Maw (*21*). The wheat-fed fryers graded somewhat higher than the corn-fed birds, as noted in table 3. There were no appreciable differences between the different groups in percentage of either dark meat or skin and subcutaneous fat. But the corn- and wheat-fed birds had a greater percentage of abdominal fat than the barley-fed fryers and roasters. This agrees with the work of Maw (*16*, *17*) and Maw et al. (*18*). There were no appreciable differences between the corn- and wheat-fed fryer groups. Apparently those receiving barley and finished in the battery carried more abdominal fat than those receiving the same grain but finished on the range. The corn-fed roasters in 1937 carried more abdominal fat than the wheat-fed roasters. Especially was this true of the roasters finished in the battery. Differences between corn- and wheat-fed birds finished on the range were not appreciable. The birds receiving barley had more light meat than those receiving corn in 1936. The same was true in 1937, but the difference was not significant.

TABLE 4.—*Average percentage distribution of fleshing in the total edible portion of fryers and roasters fed corn, wheat, and barley on range or in battery, 1936–37*

Type of bird, year, and ration	Light meat			Dark meat			Skin and subcutaneous fatty tissue			Abdominal fatty tissue		
	Range[1]	*Battery*	*Combined*	Range	*Battery*	*Combined*	Range[2]	*Battery*	*Combined*	Range[3]	*Battery*	*Combined*
Fryers (1937):												
Corn	40.26	38.90	39.58	43.40	41.73	42.57	13.24	15.16	[4] 14.20	[5] 3.59	4.21	[5] 3.90
Wheat	[6] 43.56	41.90	[7] 42.73	42.45	41.36	41.90	12.25	12.96	12.61	[8] 1.74	3.78	[9] 2.76
Barley	[10] 43.61	40.49	[10] 42.05	44.51	43.27	43.89	11.60	13.71	[11] 12.66	[5] .28	2.55	1.41
Roasters (1937).												
Corn	40.80	38.90	39.84	43.00	43.20	43.10	12.72	12.20	12.46	[5] 3.51	[5] 5.74	[5] 4.62
Wheat	40.60	40.50	49.52	45.40	44.10	44.76	12.22	12.68	12.45	1.82	[12] 2.70	[12] 2.26
Barley	42.04	40.71	41.38	44.63	44.67	44.65	12.03	12.72	12.38	1.29	1.90	1.59
Roasters (1936):												
Corn	34.49			46.35			13.96			[11] 3.18		
Wheat	36.84			45.27			14.12			[8] 3.45		
Barley	[13] 39.04			47.38			12.60			.98		

[1] The response of barley-fed fryers was significantly greater on the range than in the battery.
[2] The response of barley-fed fryers was significantly greater in the battery than on the range.
[3] The response of wheat-fed fryers was significantly greater in the battery than on the range; the response of barley-fed fryers was highly significantly greater in the battery than on the range.
[4] Significantly greater response to corn than to wheat.
[5] Highly significantly greater response to corn than to barley.
[6] Significantly greater response to wheat than to corn.
[7] *Highly significantly greater response to wheat than to corn.*
[8] Highly significantly greater response to wheat than to barley.
[9] Significantly greater response to wheat than to barley.
[10] Significantly greater response to barley than to corn.
[11] Significantly greater response to corn than to barley.
[12] Highly significantly greater response to corn than to wheat.
[13] Highly significantly greater response to barley than to corn.

Protein and ash analyses were not made on the edible portions of the cockerels grown in 1937 because it was felt that the protein and ash content probably would have no influence on the edible quality of the meat. The analyses made in 1936 of the 12 birds in each group show no significant differences among the different groups (table 5). This agrees with the report of Maw (*19*).

It should be emphasized that the standard errors of the averages for the protein, ash, moisture, and fat in the abdominal fatty tissue were very high because of the difficulty in getting sufficiently large samples from the birds. This is especially true of those receiving barley, in which group many of the birds had no abdominal fatty tissue.

TABLE 5.—*Average protein and ash content of light and dark meat and of fatty tissues of groups of 12 birds grown in 1936 and fed corn, wheat, and barley*

Ration	Light meat		Dark meat		Skin and subcutaneous fatty tissue		Abdominal fatty *tissue*	
	Protein	Ash	Protein	Ash	Protein	Ash	Protein	Ash
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Corn	24.37	1.10	21.04	1.17	21.78	0.65	4.02	0.23
Wheat	23.98	1.08	21.76	1.07	21.95	.63	3.77	.24
Barley	23.87	1.16	21.62	1.12	24.95	.80	6.22	.54

Table 6 gives the percentage of fat in the different edible portions of fryers and roasters. From these figures it is evident that the corn- and wheat-fed fryers and roasters had somewhat more fat in both the light and dark meats and more skin and subcutaneous fat than the birds fed barley. Differences between corn- and wheat-fed birds are not significant, a finding which agrees with that of Harshaw (*7*) and Maw (*17*), who report corn to be superior to other grains for fattening.

TABLE 6.—*Average fat content of edible portions of roasters and fryers (moisture-free) fed corn, wheat, and barley on range or in battery, 1936–37*

Type of bird, year, and ration	Light meat			Dark meat			Skin and subcutaneous fat			Abdominal fat		
	Range[1]	Battery	Combined	Range[2]	Battery	Combined	Range[3]	Battery	Combined	Range[4]	Battery	Combined
Fryers (1937):	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Corn	[5] 7.61	8.92	[5] 8.30	[6] 17.28	17.48	[5] 17.39	54.50	61.37	57.94	88.18	91.97	90.31
Wheat	[7] 6.67	9.77	8.14	13.59	17.18	15.29	55.74	56.34	56.03	87.90	91.00	89.67
Barley	4.47	7.05	5.76	10.61	16.33	13.47	52.94	54.28	53.57	(8)	88.28	---
Roasters (1937):												
Corn	9.32	[5] 13.18	11.12	15.39	[5] 21.56	[5] 18.47	44.19	53.79	49.84	91.20	96.07	94.23
Wheat	8.77	10.90	9.84	13.04	17.39	[9] 15.33	44.24	48.31	46.50	85.46	93.50	91.03
Barley	8.44	9.95	9.24	12.76	16.08	14.42	39.79	48.90	44.59	96.46	94.42	95.30
Roasters (1936):												
Corn	[5] 9.14	---	---	[6] 21.66	---	---	[9] 56.75	---	---	83.01	---	---
Wheat	[7] 9.06	---	---	[10] 18.66	---	---	[10] 56.82	---	---	91.69	---	---
Barley	5.90	---	---	10.15	---	---	39.23	---	---	76.60	---	---

[1] The response of barley-fed fryers was significantly greater in the battery than on the range.
[2] The response of barley-fed fryers was highly significantly greater in the battery than on the range; the response of corn-fed roasters (1937) was highly significantly greater in the battery than on the range; the response of wheat-fed roasters (1937) was significantly greater in the battery than on the range.
[3] The response of corn-fed roasters (1937) was highly significantly greater in the battery than on the range.
[4] The response of both corn-fed fryers and roasters (1937) was significantly greater in the battery than on the range.
[5] Significantly greater response to corn than to barley.
[6] Highly significantly greater response to corn than to barley.
[7] Significantly greater response to wheat than to barley.
[8] Insufficient abdominal fat present to permit chemical analysis.
[9] Significantly greater response to corn than to wheat.
[10] Highly significantly greater response to wheat than to barley.

In comparing battery and range methods of finishing, it is apparent that there was an increase in the percentage of fat in each of the four classes of edible meat when either fryers or roasters were finished in the battery. This was generally true regardless of the grain used. Significantly, and in general, the least fat was deposited in the barley-fed birds.

From table 7, it is apparent that the percentage of moisture present in the edible meat varied inversely with the percentage of fat, the barley-fed birds generally having somewhat more moisture than the corn-fed birds, with the wheat-fed birds intermediate. The inverse relationship between fat and moisture has been previously noted by

TABLE 7.—*Average moisture content of edible portions of roasters and fryers fed corn, wheat, and barley on range or in battery, 1936–37*

Type of bird, year, and ration	Light meat			Dark meat			Skin and subcutaneous fat			Abdominal fat		
	Range[1]	Battery	Combined	Range[2]	Battery	Combined	Range[3]	Battery	Combined	Range	Battery	Combined
Fryers (1937).	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Corn	73.18	74.12	73.65	72.82	72.80	72.81	50.49	51.84	51.17	9.50	9.20	9.33
Wheat	74.19	73.69	73.95	73.49	72.95	73.23	[4] 52.31	49.39	[4] 50.93	15.07	9.80	12.06
Barley	74.34	73.90	74.13	74.01	72.24	73.13	[5] 57.89	51.90	[5] 55.09	([6])	11.59	----
Roasters (1937):												
Corn	72.51	70.55	71.49	73.26	71.97	72.62	50.80	43.20	46.30	9.30	7.10	8.04
Wheat	72.15	72.75	[7] 72.96	[7] 74.88	[7] 73.79	[7] 74.33	50.20	47.50	[4] 48.70	14.40	[8] 12.50	[7] 13.20
Barley	*73.46*	[8] 73.15	[9] 73.29	[5] 74.36	[5] 73.46	[9] 73.96	55.10	[9] 52.60	[9] 53.80	[9] 19.70	[5] 12.90	[9] 15.80
Roasters (1936):												
Corn	71.65	----	----	70.44	----	----	46.77	----	----	14.22	----	----
Wheat	[4] 71.61	----	----	[10] 70.73	----	----	[10] 47.59	----	----	13.06	----	----
Barley	[5] 73.07	----	----	[9] 73.65	----	----	[5] 57.48	----	----	20.51	----	----

[1] The response of corn-fed roasters (1937) was significantly greater on the range than in the battery.
[2] The response of the barley-fed fryers was highly significantly greater on the range than in the battery, the response of the corn-fed roasters (1937) was significantly greater on the range than in the battery; the response of wheat-fed roasters (1937) was highly significantly greater on the range than in the battery.
[3] The response of barley-fed fryers was significantly greater on the range than in the battery.
[4] Significantly greater response to barley than to wheat.
[5] Significantly greater response to barley than to corn.
[6] Samples insufficient for analysis.
[7] Highly significantly greater response to wheat than to corn.
[8] Significantly greater response to wheat than to corn.
[9] Highly significantly greater response to barley than to corn.
[10] Highly significantly greater response to barley than to wheat.

TABLE 8.—*Average distribution of edible uncooked meat and of fat and moisture in the edible meat on the left halves of carcasses of fryers and roasters fed corn, wheat, and barley on range and in battery, 1936–37*

Type of bird, year, and ration	Edible meat			Fat (moisture-free basis)			Moisture		
	Range[1]	Battery	Combined	Range[2]	Battery	Combined	Range[3]	Battery	Combined
Fryers (1937):	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Corn	67.8	72.3	70.0	[4] 21.0	[5] 23.9	[4] 22.4	68.1	67.5	67.8
Wheat	70.0	70.7	70.4	[6] 17.0	21.9	[7] 19.4	70.2	67.8	69.0
Barley	68.3	69.9	69.1	[8] 12.9	19.6	[8] 15.1	72.1	68.6	[9] 70.2
Roasters (1937):									
Corn	73.9	74.5	74.2	[5] 19.2	[4] 26.5	[4] 23.0	67.9	64.2	65.9
Wheat	74.3	73.4	73.8	16.5	[10] 20.7	[10] 18.7	70.1	68.4	[11] 69.2
Barley	72.8	72.6	72.7	15.3	19.2	17.3	71.0	[12] 70.0	[12] 70.3
Roasters (1936):									
Corn	78.8	----	----	[4] 23.8	----	----	64.3	----	----
Wheat	79.7	----	----	[7] 23.1	----	----	[13] 65.7	----	----
Barley	77.3	----	----	12.8	----	----	[12] 70.8	----	----

[1] The response of corn-fed fryers was significantly greater in the battery than on the range.
[2] The response of wheat-fed fryers was significantly greater in the battery than on the range; the response of barley-fed fryers was highly significantly greater in the battery than on the range; the response of corn-fed roasters (1937) was highly significantly greater in the battery than on the range; the response of wheat-fed roasters (1937) was highly significantly greater in the battery than on the range; the response of barley-fed roasters (1937) was significantly greater in the battery than on the range.
[3] The response of barley-fed fryers was significantly greater on the range than in the battery.
[4] Highly significantly greater response to corn than to barley.
[5] Significantly greater response to corn than to barley.
[6] Significantly greater response to corn than to wheat.
[7] Highly significantly greater response to wheat than to barley.
[8] Significantly greater response to wheat than to barley.
[9] Significantly greater response to barley than to corn.
[10] Highly significantly greater response to corn than to wheat.
[11] Significantly greater response to wheat than to corn.
[12] Highly significantly greater response to barley than to corn.
[13] Highly significantly greater response to barley than to wheat.

Table 8 gives the percentage of edible uncooked meat and the percentages of fat and moisture in the left halves of edible carcasses of

fryers and roasters. The wheat- and corn-fed birds did not differ appreciably in the amount of edible meat produced, but the barley-fed birds produced less than either group. This agrees with the finding of Maw et al. (*18*). There were no appreciable differences between any of the percentages of edible meat on the left half of the carcass.

The corn-fed fryers and roasters not only had the largest total quantity of body fat, but also the largest total percentage of body fat, followed in order by the wheat- and barley-fed birds. The same relationship has been previously noted by Maw et al. (*18*).

Table 9 gives the body measurements which were taken each year. The corn-fed fryers had a somewhat greater average breast length than the wheat-fed fryers. Other body measurements did not differ appreciably among the fryers of the different lots. In the 1937 experiments the average breast width 3 inches back from the tip and the heart girth were somewhat greater in the roasters receiving corn than in those receiving barley. The tibia anterior-posterior diameter was also larger in the corn-fed than in the barley-fed birds. In the 1936 experiments, the average breast-width measurements of the wheat-fed roasters were larger than those of either the corn- or the barley-fed roasters.

TABLE 9.—*Average body measurements of fryers and roasters fed corn, wheat, and barley, range and battery groups combined, 1936–37*

Type of bird year, and ration	Breast measurements				Shoulder width	Heart girth	Hip width	Femur length	Tibia measurements		
	5/8 of an inch from anterior tip	3 inches from anterior tip	1½ inches from edge	Length					Length	Anterior posterior	Lateral width
	Centimeters	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*
Fryers (1937):											
Corn	2.64	3.07	4.04	[1] 11.73	7.70	4.45	8.00				
Wheat	2.62	3.20	3.91	[2] 11.33	7.90	4.42	8.08				
Barley	2.74	3.12	3.99	11.33	7.67	4.34	8.03				
Roasters (1937):											
Corn	[3] 2.77	[1] 3.35	[3] 4.83	13.89	[3] 9.76	[1] 5.93	9.59	12.52	17.09	[1] 5.22	3.63
Wheat	2.66	[4] 3.04	4.64	14.05	9.52	[2] 5.56	9.64	12.67	17.12	[5] 5.08	3.65
Barley	2.59	3.00	4.33	13.74	9.40	5.40	9.50	12.65	16.76	4.88	3.59
Roasters (1936):											
Corn	2.87	2.91	4.94	14.07	9.29						
Wheat	[5] 3.02	3.21	5.07	[5] 14.66	[7] 9.68						
Barley	2.69	2.68	4.87	13.54	[6] 9.08						

[1] Highly significantly greater response to corn than to barley.
[2] Highly significantly greater response to corn than to wheat.
[3] Significantly greater response to corn than to barley.
[4] Significantly greater response to corn than to wheat.
[5] Significantly greater response to wheat than to barley.
[6] Highly significantly greater response to wheat than to barley.
[7] Significantly greater response to wheat than to corn.

It is evident from table 10 that there was more total body fat in the battery-finished birds than in the birds finished on the range. This was true of all groups regardless of the cereal grain used. There was a larger amount of total fat in the corn-and wheat- fed fryers and roasters than in the barley-fed birds, whether they were finished on the range or in batteries. The fryers and roasters receiving yellow corn had the largest amounts of fat in the light meat, dark meat,

skin and subcutaneous tissue, and the abdominal fatty tissue, followed closely by those receiving wheat; those receiving barley had appreciably less fat in each of the different classes of edible meats.

TABLE 10.—*Average weight and percentage distribution of fat in the total fat of fryers and roasters fed corn, wheat, and barley on range and in battery, 1936–37*

Type of bird, year, and ration	Weight of total fat in left half of carcass		Flesh fat				Skin and subcutaneous fat				Abdominal fat			
	Range	Battery	Range		Battery		Range		Battery		Range		Battery	
	Grams	*Grams*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
Fryers (1937):														
Corn	71.7	83.3	36.0	50.2	37.6	45.0	25.1	35.0	32.5	39.0	10.6	14.8	13.3	16.0
Wheat	60.8	73.7	31.0	51.0	37.7	51.1	24.5	40.3	24.2	32.9	5.3	8.7	11.8	16.0
Barley	38.4	62.9	19.9	51.8	31.9	50.7	18.5	48.2	23.9	38.0	0	0	7.1	11.3
Roasters (1937):														
Corn	113.6	173.3	61.8	54.4	94.3	54.4	33.6	29.6	43.0	24.8	18.2	16.0	36.0	20.8
Wheat	96.7	121.2	55.8	57.7	70.4	58.1	31.9	33.0	35.8	29.5	0.0	9.3	15.0	12.4
Barley	85.7	103.7	51.8	60.5	60.5	58.3	27.1	31.6	33.3	32.1	6.8	7.9	9.9	9.6
Roasters (1936):														
Corn	162.1	----	88.8	54.8	----	----	55.0	33.9	----	----	18.3	11.3	----	----
Wheat	173.0	----	88.4	51.1	----	----	60.8	35.1	----	----	23.8	13.8	----	----
Barley	80.7	----	43.9	54.4	----	----	31.4	38.9	----	----	5.4	6.7	----	----

Relative to the distribution of the total body fat, it is apparent that as the percentage of abdominal fat increased in each group the percentage of fat in the other parts of the body decreased correspondingly. The abdominal cavity appears to be the last place in which fat is deposited. Many of the birds fed the barley ration had no abdominal fatty tissue. Had those receiving barley been fattened for a longer period than those receiving corn or wheat, however, the results might have been different. In the fryers, of all groups, from 45.0 to 51.8 percent of the total fat was deposited in the light and dark meats, 32.9 to 48.2 percent in the skin and subcutaneous fat, and 0 to 16 percent in the abdominal cavity. In the roasters, from 51.1 to 60.5 percent of the total fat was present in the light and dark flesh, 24.8 to 38.9 percent in the skin and subcutaneous tissue, and 6.7 to 20.8 percent in the abdominal fatty tissue. It is apparent that as fattening progresses fat continues to be deposited in the light and dark meats and in the skin as well as in the abdominal cavity. The amount of abdominal fatty tissue present gives a very good indication of the total amount of fat in the body.

Table 11 shows the relationships of the dressed weight to the total edible meat, the fat content, the alignment grade, and the index value. These figures are averages for the combined range and battery methods of finishing fryers and roasters. They show the general superiority in all respects of corn and wheat over barley for fryers and roasters. It will be noted that in 1937 there was appreciably more total edible meat on the carcass of the corn-fed roasters than on that of the barley-fed. The index value is also in close agreement and both differences are significant. A covariance analysis of the correlation between index value and the total edible meat on the left half of the carcass shows that for all the roasters produced in 1937 there was a positive correlation of 0.37, which is highly significant

and shows that this method of determing index value can be used in estimating the total edible meat present in the carcass, especially for roasters.

TABLE 11.—*Relationships of average carcass measurements of fryers and roasters fed corn, wheat, and barley (range and battery groups combined), 1936–37*

Type of bird, year, and ration	Dressed weight of whole carcass undrawn	Total edible meat on left half of carcass	Average fat content of left half of carcass	Alinement grade [1]	Index value
	Grams	*Grams*	*Grams*		
Fryers (1937):					
Corn	1,151	[2] 346	78	2.6	194
Wheat	1,156	[3] 348	68	2.1	194
Barley	1,074	310	47	2.8	190
Roasters (1937)					
Corn	1,912	[4] 623	143	1.6	[4] 267
Wheat	1,852	[5] 586	110	[6] 2.6	233
Barley	1,762	[2] 550	95	[7] 3.2	[2] 218
Roasters (1936).					
Corn	2,094	[8] 676	162	2.6	232
Wheat	2,195	[6] 731	173	1.9	[3] 287
Barley	1,907	[3] 623	81	[9] 3.0	197

[1] On the basis of 1.0 being the top market grade and 4.0 being the lowest market grade.
[2] Highly significantly greater response to corn than to barley.
[3] Highly significantly greater response to wheat than to barley.
[4] Significantly greater response to corn than to wheat.
[5] Significantly greater response to wheat than to barley.
[6] Highly significantly greater response to wheat than to corn.
[7] Highly significantly greater response to barley than to corn.
[8] Significantly greater response to corn than to barley.
[9] Significantly greater response to barley than to wheat.

SUMMARY

When judged by the amount of feed required to produce a unit of gain in body weight of fryers during the growing period, wheat was most efficient, followed in order by barley and corn. In growing rations for roasters, the gain-feed ratio was practically the same for corn and wheat, but somewhat more barley was required for the same gain in body weight. In the finishing rations tested, corn, wheat, and barley ranked in this order of efficiency.

There were no appreciable differences in the average rates of gain in body weight between the corn-, wheat-, and barley-fed fryers and roasters receiving the growing rations in 1937. In 1936, however, those receiving barley grew less rapidly than those receiving corn or wheat. As finishing rations, yellow corn and wheat proved superior to barley for most of the groups, slightly better gains being made by the corn-fed group than by the wheat-fed.

The fryers receiving corn or wheat in the ration and finished on the range gained somewhat more in weight than the corresponding groups finished in batteries during the last 3 weeks; the reverse was true with those receiving barley. With the roasters both the corn- and wheat-fed groups finished for 2 weeks in batteries gained appreciably more than those finished on the range; there were no significant differences between the range and battery groups receiving barley in the ration.

The corn- and wheat-fed fryers and roasters had significantly more total edible meat on the carcasses than the birds receiving barley. The wheat- and barley-fed fryers and roasters had a somewhat higher percentage of light meat in the total edible meat than those receiving

corn. This may have been due to the fact that the corn group had the highest percentage of abdominal fatty tissue in the edible meat.

The corn-fed fryers and roasters showed a consistent tendency to deposit more fat in the light meat, dark meat, skin and subcutaneous fat, and the abdominal fatty tissue than the other two groups. They were followed in order by the wheat- and barley-fed groups, with significantly more fat in the carcasses of the corn-fed than in those of the barley-fed group.

In comparing battery and range methods of finishing, an increase was found in the percentage of fat in each of the four classes of edible meat in both fryers and roasters finished in the battery. Generally speaking, this was true regardless of the grain used. In general, the least fat was deposited in the barley-fed birds.

A tendency was noted toward an inverse relationship between the fat and moisture content of edible meat. Consequently, the range groups generally had a slightly higher moisture content than the battery groups, and the corn-fed birds a somewhat lower moisture content than either the barley- or the wheat-fed birds.

There was a significant positive correlation between the amount of edible meat on the carcass and the index value determined from the breast measurements taken.

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EFFECT OF CORN, WHEAT, AND BARLEY IN THE DIET ON THE FLAVOR OF FRIED AND ROASTED CHICKENS[1]

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INTRODUCTION

Research dealing with the preparation and cooking of poultry meat is comparatively new; consequently many of our present-day methods might well be tested with a view to improvement. There are also many unsolved problems connected with obtaining accurate estimates of flavor since such estimates are based on people's opinions. It is probable that much of the research work involving flavors which has been done in the past has not included a sufficiently large number of opinions and some of the judges on tasting committees have not been sensitive enough to detect differences in flavor.

EXPERIMENTAL PROCEDURE

PREPARATION OF CHICKENS FOR FRYING

The experiments herein reported were conducted in 1937. One-half of each of 10 Barred Plymouth Rock cockerels 17 weeks of age from each of 3 experimental rations were used. The average dressed weights of all groups was 3.13 pounds and represented those birds nearest the average weight for the group from which they were selected. The three growing rations used included 59 percent of either ground yellow corn, wheat, or barley added to the basal mash mixture, the same whole grain that was used in the mash being also fed ad libitum. The rations used and the methods of finishing are given in a previous report by Poley et al. (*12*).[3] About 72 percent of the ration consumed during the growing period consisted of either yellow corn, wheat, or barley. During the 3-week finishing period 62 percent of the cereal grain was consumed in the all-mash finishing ration which was mixed with water to a thin paste.

Feed was withheld but water was provided for 24 hours before killing time, when the birds were weighed. They were then dressed, chilled at 36° F., and the dressed weights taken. Several physical measurements of the dressed carcass were also taken, as previously reported (*12*). Table 1 gives the weights of the different parts into which the birds were divided, and also the percentage of live weight and dressed and drawn weights, for each part of the fryers and roasters. There have been several reports, including those by Bird (*1*), Harshaw (*5*), Maw (*9*), and Maw et al. (*10*), giving dressing percentages, chemical composition, and cooking percentages, but none involved a study of cooking percentages for fryers.

[1] Received for publication November 20, 1939. *Paper No. 122 of the Journal Series of the South Dakota* Agricultural Experiment Station. Contribution from the Departments of Poultry Husbandry, Home Economics, and Chemistry.

[2] The writers are indebted to W. O. Wilson, assistant poultry husbandman, and R. L. Dolecek, assistant professor of physics, for their generous assistance in assembling and statistically analysing these data. Acknowledgment is also gratefully made to Oscar Olson, analyst, for his valuable laboratory assistance.

[3] Italic numbers in parentheses refer to Literature Cited, p. 190.

BLE 1.—*Average live and dressed weights, and dressing and cooking percentages of whole carcass and parts of fryers and roasters fed corn, wheat, and barley*

FRYERS

Item	Average dressed weights of carcasses and of parts of birds fed—			Proportion average dressed weight bore to—: Live weight of birds fed—			Dressed weight of birds fed—			Drawn weight (uncooked) of birds fed—		
	Corn	Wheat	Barley	Corn	Wheat	Barley	Corn	Wheat	Barley	Corn	Wheat	Barley
	Grams	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
rage live weight	1,640.6	1,654.0	1,577.0	100.0	100.0	100.0	113.5	113.5	116.0	140.4	142.2	147.2
rage dressed weight	1,445.9	1,456.8	1,359.2	88.1	88.1	86.2	100.0	100.0	100.0	123.7	125.3	126.9
rage drawn weight	1,168.9	1,162.9	1,071.4	71.2	70.3	67.9	80.8	79.8	78.8	100.0	100.0	100.0
lible parts:												
Head	65.0	69.0	62.9	4.0	4.2	4.0	4.5	4.7	4.6	5.6	5.9	5.9
Shanks and feet	92.0	89.1	85.5	5.6	5.4	5.4	6.4	6.1	6.3	7.9	7.7	8.0
Entrails	120.0	125.2	136.6	7.3	7.6	8.7	8.3	8.6	10.1	10.3	10.8	12.7
Total	277.0	283.3	285.0	16.9	17.1	18.1	19.2	19.4	21.0	23.7	24.4	26.6
ble carcass, uncooked: [1]												
Gizzard	40.4	33.4	36.2	2.5	2.0	2.3	2.8	2.3	2.7	3.5	2.9	3.4
Heart	6.6	6.4	5.9	.4	.4	.4	.5	.4	.4	.6	.6	.6
Liver	26.0	24.2	26.4	1.6	1.5	1.7	1.8	1.7	1.9	2.2	2.1	2.5
Neck [1]	91.3	94.5	77.9	5.6	5.7	4.9	6.3	6.5	5.7	7.8	8.1	7.3
Abdominal fat	24.2	20.9	10.5	1.5	1.3	.7	1.7	1.4	.8	2.1	1.8	1.0
Right half [1]	499.0	501.7	469.0	30.4	30.3	29.7	34.5	34.4	34.5	42.7	43.1	43.8
Left half [1]	481.4	481.8	445.5	29.3	29.1	28.2	33.3	33.1	32.8	41.2	41.4	41.6
half, uncooked:												
Light meat	136.2	148.9	132.1	8.3	9.0	8.4	9.4	10.2	9.7	11.7	12.8	12.3
Dark meat	146.7	143.2	135.7	8.9	8.7	8.6	10.1	9.8	10.0	12.6	12.3	12.7
Skin and subcutaneous fat	50.1	44.0	40.9	3.1	2.7	2.6	3.5	3.0	3.0	4.3	3.8	3.8
Bones	143.8	140.6	127.4	8.8	8.5	8.1	9.9	9.7	9.4	12.3	12.1	11.9
Moisture loss during dissection	4.6	5.1	9.4	.3	.3	.6	.3	.4	.7	.4	.4	.9
Total	481.4	481.8	445.5	29.3	29.2	28.3	33.3	33.1	32.8	41.3	41.4	41.6
it half, uncooked	470.2	476.2	450.9	28.7	28.8	28.6	32.5	32.7	33.2	40.2	40.9	42.1
it half, cooked:												
Light meat	113.7	121.8	106.6	6.9	7.4	6.8	7.9	8.4	7.8	9.7	10.5	9.9
Dark meat	113.1	114.8	110.9	6.9	6.9	7.0	7.8	7.9	8.2	9.7	9.9	10.4
Skin and subcutaneous fat	29.6	30.4	27.1	1.8	1.8	1.7	2.0	2.1	2.0	2.5	2.6	2.5
Drippings	21.2	19.3	19.9	1.3	1.2	1.3	1.5	1.3	1.5	1.8	1.7	1.9
Total edible	277.6	286.3	264.5	16.9	17.3	16.8	19.2	19.7	19.5	23.7	24.7	24.7

Bones and inedible	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	8.3
Total edible and inedible	370.3	378.9	352.9	22.6	22.9	22.4	25.6	26.1	26.0	31.6	32.7	33.0
Cooking loss	99.9	97.3	98.0	6.1	5.9	6.2	6.9	6.6	7.2	8.6	8.2	9.1
ROASTERS												
erage live weight	2,633.2	2,564.5	2,469.5	*100.0*	*100.0*	100.0	*113.0*	114.0	114.0	136.8	139.7	140.3
erage dressed weight	2,329.3	2,248.8	2,165.5	88.5	87.7	87.7	100.0	100.0	100.0	121.0	122.5	123.0
erage drawn weight	1,925.1	1,836.0	1,760.4	73.1	71.6	71.3	82.6	81.6	81.3	100.0	100.0	100.0
edible parts:												
Head	97.2	95.1	90.8	3.7	3.7	3.7	4.2	4.2	4.2	5.0	5.2	5.2
Shanks and feet	123.4	126.5	119.5	4.7	4.9	4.8	5.3	5.6	5.5	6.4	6.9	6.8
Entrails	182.1	192.7	187.3	6.9	7.5	7.6	7.8	8.6	8.6	9.5	10.5	10.6
Total	402.7	414.3	397.6	15.3	16.2	16.1	17.3	18.4	18.4	20.9	22.6	22.6
lible carcass, uncooked:[1]												
Gizzard	46.9	47.4	49.0	1.8	1.8	2.0	2.0	2.1	2.3	2.4	2.6	2.8
Heart	13.0	11.7	11.0	.5	.5	.4	.6	.5	.5	.7	.6	.6
Liver	35.6	37.6	38.2	1.4	1.5	1.5	1.5	1.7	1.8	1.8	2.0	2.2
Neck[1]	151.8	146.7	143.5	5.8	5.7	5.8	6.5	6.5	6.6	7.9	8.0	8.2
Abdominal fat	56.1	29.3	17.2	2.1	1.1	.7	2.4	1.3	.8	2.9	1.6	1.0
Right half[1]	823.6	794.3	755.0	31.3	31.0	30.6	35.4	35.3	34.9	42.8	43.3	42.9
Left half[1]	798.1	769.0	746.5	30.3	30.0	30.2	34.3	34.2	34.5	41.5	41.9	42.4
eft half, uncooked:												
Light meat	243.9	235.9	226.9	9.3	9.2	9.2	10.5	10.5	10.5	12.7	12.8	12.9
Dark meat	275.5	261.6	249.5	10.5	10.2	10.1	11.8	11.6	11.5	14.3	14.2	14.2
Skin and subcutaneous fat	75.2	73.0	67.8	2.9	2.8	2.7	3.2	3.2	3.1	3.9	4.0	3.9
Bones	185.8	186.7	188.2	7.1	7.3	7.6	8.0	8.3	8.7	9.7	10.2	10.7
Moisture loss during dissection	17.7	11.8	14.1	.7	.5	.6	.8	.5	.7	.9	.6	.8
Total	798.1	769.0	746.5	30.5	30.0	30.2	34.3	34.1	34.5	41.5	41.8	42.5
ight half, uncooked	836.4	809.5	771.8	31.8	31.6	31.3	35.9	36.0	35.6	43.4	44.1	43.8
ight half, cooked:												
Light meat	294.6	291.7	272.6	11.2	11.4	11.0	12.6	13.0	12.6	15.3	15.9	15.5
Dark meat	213.6	201.7	197.5	8.1	7.9	8.0	9.2	9.0	9.1	11.1	11.0	11.2
Skin and subcutaneous fat	63.4	60.1	58.9	2.4	2.3	2.4	2.7	2.7	2.7	3.3	3.3	3.3
Drippings	31.4	15.8	23.6	1.2	.6	1.0	1.3	.7	1.1	1.6	.9	1.3
Total edible	603.0	569.3	552.6	22.9	22.2	22.4	25.8	25.4	25.5	31.3	31.1	31.3
Bones and inedible	160.2	154.6	152.3	6.1	6.0	6.2	6.9	6.9	7.0	8.3	8.4	8.7
Total edible and inedible	763.2	723.9	704.9	29.0	28.2	28.6	32.7	32.3	32.5	39.6	39.5	40.0
Cooking loss	73.2	85.6	66.9	2.8	3.4	2.7	3.2	3.7	3.2	3.8	4.6	3.8

[1] Includes bones.

The weights of edible meat which were taken included the gizzard, heart, liver, and abdominal fat. The weight of the neck, which included the skin, flesh, and bone, and the weight of the right and left halves of the edible carcass were also determined. Analyses were made on the left half of the carcass for both the fat and moisture content of the edible light meat, dark meat, skin and subcutaneous fat, and abdominal fat. The results of these analyses have been reported by Poley et al. (*12*). Figure 1 shows how the carcass was cut longitudinally through the vertebrae and sternum into two halves of approximately equal weight. The right half of each carcass was placed in an individual airtight oil-silk bag and stored at a temperature of 32° to 38° F. until cooking time. When cooked, the quantities of edible light meat, dark meat, skin and subcutaneous fat, and bones were determined. The light and dark meats were then scored for palatability by a committee of judges. Two halves from each of the three rations were fried on each of 5 days, over a 14-day period.

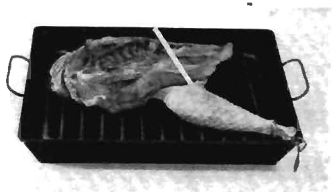

FIGURE 1.—The pan, rack, position of thermometer, and roaster during cooking.

For each chicken, 1 pound of fresh hydrogenated lard was placed in an iron chicken fryer (10 inches in diameter and 3 inches deep) and heated by electricity to 175° C. This was just enough fat to bubble up over the chicken and give all the surfaces a golden-brown crust. Thermometers were placed in the thigh and breast pieces, but since there was insufficient flesh to protect the thermometer bulbs from the hot fat, the temperature readings were unreliable and higher than the "doneness" of the meat would indicate; so each chicken was cooked in the uncovered pan for 30 minutes. The temperature of the fat usually dropped about 30° from the original reading within 5 minutes after the chicken was placed in the fryer and remained at about 145° throughout the rest of the cooking period. The average weights of the right halves of the 10 chickens selected from each ration were 470 gm. for the corn-fed, 476 gm. for the wheat-fed, and 451 gm. for the barley-fed birds. The half to be fried was cut into two parts, dark and light meat. The former included the tibia, metatarsus, and flesh over the ilium (often called the "oyster"); the latter included the breast muscle and wing. No seasoning, flour, or other substance that might affect the flavor of the meat was used. The skin was not employed in the flavor studies, although it was left on the carcass until frying was completed.

PREPARATION OF CHICKENS FOR ROASTING

Ten 26½-week-old Barred Plymouth Rock cockerels were selected from each 17-bird group finished. The average weights of these birds (table 1), were closest to the average weight of the group from which they were taken. The birds had received the same growing and finishing rations that were fed to the fryers and were selected in the same manner.

About 82.9 percent of the ration consumed by the corn-fed group during the growing period consisted of yellow corn. Eighty-nine percent of the ration consumed by the wheat-fed group was wheat, and 83.6 percent of that consumed by the barley-fed group was barley. During the 2-week finishing period, 62 percent of either yellow corn, wheat, or barley was consumed, as this percentage of grain was included in the all-mash finishing ration which was mixed with water to a thin paste just before feeding.

The roasters were killed after being deprived of feed for 24 hours, with water to drink the first 12 hours, and then were dissected. The different parts into which the carcass was separated are given in table 1. The left half was used for chemical analysis and the right half for palatability studies, the same method being used as with fryers.

The average dressed weights of the 10 halves of the birds from each ration were 2,329.3, 2,248.8, and 2,165.5 gm. respectively for the corn, wheat, and barley-fed roasters. The birds were roasted with the skin side down on racks in pans (fig. 1) in an automatically controlled electric oven large enough for six pans. A meat thermometer was inserted in the thigh muscle and the temperature allowed to reach 92° C. The oven temperature was maintained at about 149° C. Niles (*11*) reported that the internal temperature of the thigh should reach 85° C. Moreover, she reported that the flavor of chicken roasted in an uncovered pan was preferable, although the skin was dry as compared to that which was roasted in a pan covered for part of the time. Lowe (*7*) also reported that the internal temperature of the thigh muscle of chicken should reach 85°. In the present experiments, the flesh did not appear sufficiently cooked at 85°, so 92° was preferred. Maw (*8*) used a constant oven temperature of 191° C. for roasting chicken.

Two oven thermometers were used inside of the oven to check the temperatures, which were read every 10 minutes. Two chickens from each of the rations were roasted at one time on each of 5 days within a 10-day period.

As soon as the temperature of the thigh muscle reached 92° C. the carcass was taken from the oven and weighed. The skin was then removed and samples taken for palatability studies. The same method used in determining the weights of the bones and edible flesh of fryers was employed. The residue included the juice which dripped from the carcass during roasting and the juice which drained from the carcass when the skin and subcutaneous fat were removed and the leg and thigh were separated from the remainder of the carcass.

FLAVOR STUDIES

Palatability studies were made on both the light and dark meats, and consisted of scoring as to intensity and desirability for both fryers and roasters. The intensity measurements were subdivided into aroma, flavor, juiciness, texture, and tenderness. Texture was judged only for the roasters. Desirability was subdivided into aroma, flavor, and juiciness. These palatability factors were evaluated from 1 to 7, according to the increasing intensity and desirability of each factor. This was done independently by individuals of a committee of nine judges according to a grading chart for cooked meat similar to the one used in the National Cooperative Project of Meat Investigations,[4] except that no consideration was given to the intensity and desirability of fat flavor. Throughout the test, each judge was served the same cross section of muscle from each chicken, with samples taken from both light and dark meats. The samples were cut as quickly as possible at right angles to the length of the muscle, and served while still warm to each judge. The judges were allowed water and white bread but no butter. No salt or other seasoning was used. As soon as the judges had completed the scoring of light and dark meats from one carcass, they were given samples from each of the five remaining carcasses. The six samples (12 portions) were distributed at random among the judges. Consequently the judges had no knowledge of the classification of the birds from which the samples were taken.

TESTING THE JUDGES

Crocker and Platt (*3*) state that there are four commonly accepted kinds of taste buds which give the four primary tastes, sweet, sour, salty, and bitter. Since it has been demonstrated by a number of workers (*2*, *4*, *13*) that considerable variation exists among individuals in their response to taste and smell, and some people cannot satisfactorily detect certain flavors, tests were made to determine the sensitivity and consistency of response of the nine judges who assisted in the study. The method used was similar to the one described by King (*6*), with one modification; namely, the solutions of sucrose (sweet), sodium chloride (salt), lactic acid (sour), and caffeine (bitter) were given in random order. This was done to eliminate the possibility of psychological reactions to the solutions of low concentration. The solutions and concentrations used are shown in table 2.

TABLE 2.—*Solutions and molar concentrations of solute used in determining the sensitivity and consistency of response of the nine judges*

Solution No.	Sucrose	Sodium chloride	Lactic acid	Caffeine
	Moles	*Moles*	*Moles*	*Moles*
1	0.0005	0.0010	0.0001	0.0001
2	.0010	.0020	.0002	.0002
3	.0025	.0050	.0005	.0005
4	.0050	.0100	.0010	.0010
5	.0100	.0500	.0025	.0025
6	.0250	.1000	.0050	.0050
7	.0500	.2000	.0100	.0100
8	.1000	.2500	.0250	.0250
9	.1500			

[4] U. S. BUREAU OF ANIMAL INDUSTRY. A STUDY OF THE FACTORS WHICH INFLUENCE THE QUALITY AND PALATABILITY OF MEAT. Rev. ed., 76 pp. 1927.

Without a knowledge of either the kind or the concentration of the solution used, each judge was required to identify the solution and estimate its concentration The following numbers and descriptive terms were employed: 0, No taste; 1, very faint; 2, faint; 3, easily distinguishable; 4, strong; 5, very strong. The judges were given two tests approximately 6 weeks apart, the same procedure being used in both.

The ability to recognize a low concentration of a solution as well as to estimate consistently the intensity of a taste are essential to good judging of flavor. The range of molar concentrations for the judges' threshold of taste as well as the median molar concentration of the solutions are given in table 3. (The threshold value of taste is the lowest molar concentration at which a taste can be identified. This is qualified by the requirement that all higher concentrations be accurately identified).

The values shown in table 3 compare favorably with the values determined by King (*6*). The frequency distribution of threshold values for the nine judges approximates a normal curve. One judge was found to have uniformly low threshold values for all substances, one judge had uniformly high threshold values, two judges had uniformly average threshold values, and the threshold values of the remaining judges were mixed.

TABLE 3.—*Median and range of taste thresholds of the nine judges*

Substance	Solution concentration at threshold		Substance	Solution concentration at threshold	
	Median	Range		Median	Range
	Moles	*Moles*		*Moles*	*Moles*
Sucrose	0.0153	0.0025–0.05	Lactic acid	0.0025	0.0002–0.005
Sodium chloride	.0550	.005 – .20	Caffeine	.0019	.001 – .005

The term "consistency" denotes the ability of a judge to reproduce his estimates of taste intensity. To determine the consistency of a judge, the difference in the identification numbers of the solutions representing his threshold values on test 1 and test 2 were used. For example, if on test 1 a judge first identified sucrose (threshold value) at a concentration of 0.0010 mole, and on test 2 he identified sucrose at a concentration 0.0025, his consistency number for sucrose would be $3-2=1$. To further estimate the consistency of a judge, the difference in the numbers of the solutions representing his choice of "easily distinguishable taste" was used. The results are shown in table 4. An examination of the distribution of consistency numbers in table 4 shows that the judges were able to reproduce responses more accurately at the easily distinguishable than at the threshold level of taste. This observation substantiates the finding of King (*6*). The average consistency number for the committee of judges at the threshold level of identification was 1.4 (range 0.6–2.0) and for the easily distinguishable level of identification 0.99 (range 0.3–1.7). These consistency numbers compare favorably with those of the 14 judges selected by King from a group of 64 judges.

TABLE 4.—*Consistency numbers of the nine judges of flavor*

Average consistency numbers	"Threshold" distribution	"Easily distinguishable" distribution
0.0–0.3	0	1
.3– .9	3	4
.9–1.5	4	4
1.5–2.1	2	0

To summarize, it is believed that the judges who assisted in these meat-tasting studies represented an average or better than average group as judged by the data of King (*6*). It is, therefore, assumed that their estimates of meat quality are reliable.

STATISTICAL ANLAYSES

Statistical treatment included an analysis of variance. *F* values were calculated and compared to the minimum *F* value necessary for significance as reported by Snedecor (*14, table XXXV*). *F* values necessary for odds of 19 to 1 (significant) and 99 to 1 (highly significant) are reported. The *F* value was determined by the following formula: $F=\frac{\text{larger mean square}}{\text{smaller mean square}}$. For example, for intensity of aroma of the light-meat samples (table 6), an analysis of variance of the palatability scores was made as shown in table 5. Where the *F* value approached significance, a more complete analysis was made with a view to subtracting out the sum of squares between birds and the sum of squares between samples. This analysis did not raise any of the mean differences to the level of high significance (odds 99 to 1).

TABLE 5.—*Analysis of variance of the palatability score for aroma of light meat in table 6*

Source of variation	Degrees of freedom	Sum of squares	Mean square	*F* value
Total	219	476.10		
Between means of feed	2	.68	0.34	0.2
Within feeds	217	475.42	2.19	

EXPERIMENTAL RESULTS

FRYERS

The dressing percentages of the different parts into which the carcasses were separated are given in table 1. The table shows no appreciable differences in dressing percentages between the birds receiving the different grains in their rations. However, there was a tendency for the barley-fed group to have slightly lower percentages of their live weight as dressed and drawn weights than the corn-and wheat-fed birds, which were about equal. There was also a slightly higher percentage of inedible and a lower percentage of total edible meat on the carcasses of the barley-fed fryers as compared to those receiving either corn or wheat, although none of these differences were significant.

There were no appreciable differences between the cooking losses of either the barley- or the wheat-fed birds as compared with those of the birds receiving yellow corn in the ration. There was slightly less cooked light meat, dark meat, and skin and subcutaneous fat on the barley-fed fryers than on the fryers receiving the other grains. This is to be expected in view of the lower body weights of these barley-fed birds. It should be noted that the percentages which are given for the uncooked and cooked parts of the left and right halves are based only on half of the carcass. In order to determine the percentage of either the edible meat or bones for the whole carcass, the following formula may be used:

$$A=2\frac{B}{C}$$

Where A=the percentage of either live weight, dressed, or drawn weight; B=the average weight of either the light meat, dark meat, skin and subcutaneous fat, or bones; and C=the average weight of the birds either alive, dressed, or drawn. Thus, the percentage of edible light meat on the whole dressed carcass for the corn-fed fryers would be calculated as follows:

$$\text{Percentage of edible light meat}=\frac{2\times 136.2}{1,445.9}=18.8\text{ percent.}$$

With the corn-fed fryers, it may be calculated that 19.4 percent of the whole uncooked drawn carcass was cooked light meat, while the same percentage was dark meat, with 5 percent skin, 3.6 percent drippings (total 47.4 percent edible), and 15.8 percent cooked bones and inedible. The cooking loss based upon the drawn weight of the uncooked carcass was 17.0 percent. It should be kept in mind that these percentages include only the two halves. The neck, abdominal fat, and giblets were not cooked. The two halves, however, represented 81.4 percent of the edible carcasses of the birds receiving yellow corn. The cooking percentages for those receiving barley were approximately the same as for the corn-fed group.

TABLE 6.—*Mean palatability scores for light and dark meat of fryers and roasters fed corn, wheat, and barley*

FRYERS

Ration		Intensity factors								Desirability factors					
		Aroma		Flavor		Tenderness		Juiciness		Amora		Flavor		Juiciness	
	Birds	Light [1]	Dark [1]	Light [2]	Light [2]	Light [3]	Dark [4]	Light [5]	Dark [5]	Light [6]	Dark [6]	Light [7]	Dark [8]	Light [9]	Dark [10]
	Number														
Corn	10	3.87	4.30	4.26	4.54	5.09	5.20	3.53	4.03	3.63	4.08	3.46	4.21	3.61	4.06
Wheat	10	3.76	3.89	4.50	4.38	4.67	5.10	3.61	4.12	4.03	4.10	4.18	4.47	4.00	4.31
Barley	10	3.81	4.07	4.30	4.44	5.19	5.05	3.54	3.98	3.75	3.86	4.11	4.10	3.71	4.16
F value [11]		.2	2.8	1.6	2.7	1.5	.5	.2	.5	1.2	.2	2.1	.5	.5	.5

See footnotes at end of table.

TABLE 6.—*Mean palatability scores for light and dark meat of fryers and roasters fed corn, wheat, and barley*—Continued

ROASTERS

Ration		Intensity factors										Desirability factors					
		Aroma		Texture		Flavor		Tenderness		Juiciness		Aroma		Flavor		Juiciness	
	Birds	Light [12]	Dark [12]	Light [13]	Dark [13]	Light [14]	Dark [2]	Light [15]	Dark [4]	Light [16]	Dark [17]	Light [18]	Dark [19]	Light [20]	Dark [21]	Light [22]	Dark [23]
Corn	16	4.59	4.76	5.81	5.33	5.24	5.08	6.16	5.41	5.10	4.90	5.13	4.93	5.29	5.01	5.20	4.82
Wheat	17	4.79	4.91	6.00	5.30	5.25	5.01	6.07	5.15	5.09	4.73	5.33	4.92	5.30	4.93	5.11	4.84
Barley	15	4.69	4.73	5.85	5.30	5.14	4.94	6.06	5.04	5.26	4.92	5.03	4.64	5.18	4.47	5.23	4.78
F value [24]		.7	.7	1.1	.5	.6	.1	.3	2.2	.3	.6	1.4	.9	.1	2.3	.1	.3

[1] Perceptible to slightly pronounced aroma.
[2] Slightly to moderately pronounced flavor.
[3] Slightly tough to moderately tender.
[4] Moderately tender to tender.
[5] Perceptible to slightly rich juciness.
[6] Neutral to slightly desirable aroma.
[7] Neutral to slightly desirable flavor.
[8] Slightly to moderately desirable flavor.
[9] Dry to slightly dry.
[10] Slightly rich to moderately rich in juiciness.
[11] For the difference between the means in any column to be significant the *F* value entered below the column must equal or exceed 3.04, for the difference to be highly significant the *F* value must equal or exceed 4.71.
[12] Slight to moderately pronounced aroma.
[13] Moderately fine to fine texture.
[14] Moderately pronounced to pronounced flavor.
[15] Tender to very tender.
[16] Moderately rich to rich juiciness.
[17] Slight to moderately rich juiciness.
[18] Moderately desirable to desirable aroma.
[19] Slight to moderately desirable aroma.
[20] Moderately desirable to desirable flavor.
[21] Slight to moderately desirable flavor.
[22] Moderately juicy to juicy.
[23] Slightly dry to moderately juicy.
[24] Significant *F* value, 3.21; highly significant *F* value, 5.11.

Palatability scores given in table 6 show that there were no significant differences in the intensity or desirability factors in respect to aroma, flavor, tenderness, or juiciness of the light and dark meats from the fryers receiving either corn, wheat, or barley as the principal constituent of the ration. The greatest differences in the judges' scores are found in (1) the intensity of aroma of the dark meat of the corn-fed fryers, which was scored slightly higher than that of either the wheat- or barley-fed fryers, and (2) tenderness, which was scored slightly higher in the corn-fed than in the wheat-fed. Of the desirability factors, the aroma and juiciness of the light meat of the wheat-fed groups were scored slightly higher than for either the corn- or barley-fed birds. The flavor of the light meat was also scored a little higher with those receiving corn. None of these differences was significant, however.

It is worthy of note that Satorius and Child (*13*) found with beef that there was a high positive correlation between judges' tenderness scores and shear force required to cut muscles. This fact indicates that a judge can estimate tenderness with accuracy without the aid of a mechanical shearing device. No positive correlation, however, was found between the quantity of press fluid, or ether extract of fat, and the palatability-juiciness character as scored by the judges. Other

palatability factors such as flavor-aroma which stimulate the flow of saliva were found to be more important in affecting the judges' score of juiciness than the fat content or the amount of press fluid.

ROASTERS

The dressing and cooking percentages of the edible and inedible parts of the carcasses of roasters were not appreciably different whether the birds received yellow corn, wheat, or barley, as will be noted from table 1. As with the fryers, the average dressed weight was appreciably less for the barley-fed groups than for the corn-fed. The birds receiving wheat also weighed less than those receiving corn. The percentage dressed weight of the live weight was somewhat greater with the birds receiving corn, as would be expected with slightly better fleshing. The percentage of total inedible parts was also slightly higher and the percentage of edible meat slightly lower in these birds than in those receiving either barley or wheat. There were no appreciable differences between the percentages of edible cooked light flesh, dark flesh, and skin, although the corn-fed birds had somewhat larger amounts, followed in order by those fed wheat and barley. There was a somewhat greater residue of drippings and juice from the corn-fed birds, less from the barley-fed, and least from the wheat-fed. The inedible portion was slightly higher in the corn group, but the percentages were practically the same for all groups. Cooking losses varied a great deal within the groups, but seemed slightly higher with those receiving wheat. All of these calculations are based upon half of the carcass and do not include the giblets, abdominal fat, or neck. To calculate the percentages for the whole carcass, the formula given for the fryers is used. Accordingly, 30.6 percent of the drawn weight was light meat in the corn-fed roasters while 22.2 and 6.6 percent was dark meat and skin respectively (total 59.4 percent edible), with 3.2 percent residue or drippings and 7.6 percent cooking loss by evaporation. These percentages might be expected if roasters are cut in half for roasting; naturally they do not indicate the percentages that would be obtained if other roasting methods were used.

The palatability scores for roasters given in table 6 show that there were no significant differences in either the intensity or desirability factors as regards aroma, flavor, tenderness, and juiciness of the light and dark meats from the birds receiving corn, wheat, or barley as the principal constituent of the ration. The differences between the average scores of the birds in the different roaster groups were even smaller than the differences between the fryers on the different rations.

The greatest differences between the average scores of the judges were in tenderness and desirability of flavor of the dark meat. The dark meat of the corn-fed roasters was considered slightly tenderer and of better flavor than that of the barley-fed roasters. In general, there was considerable variation in the judges' scores, and therefore agreement could not be reached on whatever differences might have existed in the palatability of these roasters. It should be kept in mind that, although all these birds were fed, managed, dressed, drawn, chilled, and roasted under uniform conditions there may have been some differences due to handling that affected palatability. For instance, if the bird did not bleed completely when killed, any blood remaining in the tissues might, after a few days holding, taste rather strong and affect the judges' score of the flesh. However,

precautions were taken in an effort to get as complete drainage of the blood as possible.

CONCLUSIONS

There were no significant differences between the dressing and cooking percentages of the fryers and roasters receiving either wheat or barley as the principal constituent of the ration and those receiving yellow corn.

A committee composed of nine judges could detect no appreciable differences in aroma, flavor, juiciness, or tenderness of either the light or dark meat from fryers and roasters receiving either corn, wheat, or barley in the growing and finishing rations.

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THE INFLUENCE OF INTENSITY OF EGG PRODUCTION UPON INFERTILITY IN THE DOMESTIC FOWL[1]

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INTRODUCTION

The eggs produced by breeding flocks of apparently normal hens under systems of good management include various proportions which are infertile, the number sometimes ranging as high as 30 percent (*15*)[2] of the total incubated. With small numbers of birds or under adverse conditions much larger proportions of the eggs laid may be infertile. The causes of much, perhaps most, of this infertility remain unknown and additional knowledge concerning their control is essential to an adequate understanding of avian reproduction and to the diminution of such economic losses as now result from the incubation of infertile eggs.

The objects of the present experiment were (1) to determine whether degree of infertility is influenced by the intensity of egg production in the fowl, and (2) to study the bases for such a relationship if it exists.

MATERIALS AND METHODS

During the 5-year period, 1935 to 1939, more than 1,200 females and more than 100 males representing several different strains of White Leghorns were used for this study. These hens do not constitute a random sample since those previously shown by individual performance to be poor layers were not placed in the breeding pens. They are, therefore, comparable to better-than-average commercial breeding flocks and are excellent material for study of the practical aspects of infertility.

A study of this nature is so complicated by various causes of infertility which are unknown or cannot be standardized, that the discovery of new facts bearing on the problem is possible only by the use of very large numbers of birds and eggs. Even with the large numbers considered in this study, a very few hens with excellent records of production have caused disconcerting irregularities in the results because all, or nearly all, of their eggs were infertile from causes unknown, but probably not related to intensity of egg production. Such hens have nevertheless been included in this study because there is apparently no level of infertility above which it can be said that intensity of egg production exerts no influence.

Artificial illumination was not used in the breeding pens until the January preceding the incubation season (February, March, and April) except in special matings reported in table 5. Matings were made in pens having 1 male with 12 to 16 females.

[1] Received for publication January 6, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 205.

The number of eggs laid, as given in tables 1, 2, and 3, is assumed to equal the number of eggs incubated. It is therefore subject to the usual small error in trap-nesting and marking the eggs, plus an error due to loss and breakage prior to incubation. Since 24,000 to 40,000 eggs are considered in tables 1 to 3, it is believed that such errors would be distributed by chance and therefore not materially influence results, except perhaps in measurements involving size of clutch.

Infertility was determined by candling the eggs (tables 1–4), or by appearance of the germinal disk of the broken egg after 18 to 25 hours of incubation. By neither of these methods is one able to distinguish between infertile eggs and ones with embryos dead at very early stages, but there is a practical limit to the embryonic age at which fertility can be routinely determined. With the large number of hens involved in this study such errors are assumed to occur at random.

EXPERIMENTAL RESULTS

RELATION BETWEEN FERTILITY AND INTENSITY OF PRODUCTION

The period studied each year extended from February 1 to April 30, the normal incubation season. By such a restriction one avoids the extreme fluctuation in the rate of production from season to season. The frequent occurrence of such variations suggests that any accurate measure of intensity must be based upon the egg production during a relatively short period of time. Individual incubation records for each hen were obtained for eggs laid during February, March, and April of 1935 to 1939, inclusive, and were studied by the use of the following three measures of intensity of egg production: (1) The number of eggs laid during a specified period of 6 weeks; (2) the number of eggs laid during each week of the incubation period; and (3) the number of eggs laid per clutch (number of eggs laid on consecutive days).

Number of Eggs Laid During a 6-Week Period

The number of eggs laid during a 6-week period was tabulated for each of 856 females. During the 5 years this 6-week period fell between February 22 and April 14. Slight differences occurred within these limits from year to year because of shifts in the hatching schedule. The periods did not include the first and last hatches of the season, and periods for all hens during a given year were identical. Those hens which did not lay during each of the 6 weeks were not included, because a true measure of their intensity would not have been obtained.

Egg production by different individuals during the 6-week period ranged from 13 to 40 eggs, the mode falling between 29 and 33 eggs. It is clear that those hens which laid the greater number of eggs laid relatively fewer infertile eggs (table 1). In each of the 5 years, eggs from those hens which laid from 13 to 22 eggs during the 6-week test period included the highest proportion of infertiles. In 3 of the 5 years, those hens which laid the most eggs (32 to 40) had proportionately fewer infertile eggs than did the poorer layers. When the percentages for the 5 years are averaged, the group of females which laid the fewest eggs is shown to have nearly twice as many infertiles (24.4 percent) as the birds in the group of best layers (13.0 percent). Because there are wide differences in the proportions infertile among eggs laid during different years, unweighted averages of the percentages obtained

during the different years have been used in this study. This practice was employed to avoid giving unequal weight to those factors that influence infertility differently in different years.

TABLE 1.—*The proportion of infertile eggs produced by three groups of hens that laid 13–22, 23–31, and 32–40 eggs, respectively, during a 6-week period, 1935–39*

Year	Data for hens laying—								
	13 to 22 eggs in 6 weeks			23 to 31 eggs in 6 weeks			32 to 40 eggs in 6 weeks		
	Hens	Eggs laid	Infertile eggs	Hens	Eggs laid	Infertile eggs	Hens	Eggs laid	Infertile eggs
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
1935	16	320	26.2	102	2,860	18.2	55	1,844	9.9
1936	11	208	41.8	73	2,069	26.8	68	2,301	18.4
1937	34	657	17.8	125	3,460	12.4	41	1,367	11.2
1938	10	200	12.0	93	2,674	8.0	48	1,597	8.8
1939	15	288	24.3	104	2,922	12.6	61	2,046	16.4
Total	86	1,673	[1] 24.4	497	13,985	[1] 15.6	273	9,155	[1] 13.0

[1] Unweighted average of the percentages for the 5 years.

NUMBER OF EGGS LAID DURING A 1-WEEK PERIOD

The number of eggs laid during 1 week is a good measure of intensity because such a short period of time excludes some error resulting from fluctuation of intensity during a longer period. Such error would be important if intensity of egg production influenced fertility in those eggs currently produced.

The incubation record of each hen was divided into weekly periods, each of which was assigned to one of seven groups according to the number of eggs (one to seven) laid during that week. The first week of the incubation record was omitted in each case in order to insure adequate time for mating after the males were placed in the pens, and some of the later hatches were omitted to avoid lack of uniformity in the length of the hatching season. Eggs included in these data were laid during February, March, and April. A separate study of the data for 1937 gave no indication that seasonal differences in infertility (*14*) influence the distribution of infertility shown in table 2.

TABLE 2.—*The proportion of infertile eggs produced by 1,084 hens during weekly periods in which from 1 to 7 eggs were laid, 1935–39*

Year	Infertile eggs produced by hens laying the indicated number of eggs during each week							Weighted averages (all weeks)
	1	2	3	4	5	6	7	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1935	32.5	24.2	25.0	17.9	15.5	14.1	9.2	16.2
1936	37.9	35.1	27.6	27.7	22.0	20.0	18.4	22.1
1937	31.9	23.9	12.2	16.0	11.2	10.4	3.3	12.0
1938	23.3	20.8	17.4	12.4	9.8	6.3	16.3	10.0
1939	30.3	22.6	15.7	13.6	10.8	13.7	11.7	12.9
Average (unweighted)	31.2	25.3	19.6	17.5	13.9	12.9	11.8	14.7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Total number of eggs	212	746	2,073	6,036	14,490	11,442	1,533	36,532

The proportion of infertile eggs in each of the seven groups in each of the 5 years is shown in table 2. In each year the highest proportion of infertiles was found in the group of eggs laid at the rate of only one per week, whereas the smallest proportion was found in the groups that were laid at the rapid rate of five or more eggs per week. It can be seen that there are wide differences from year to year which are probably the result, directly or indirectly, of environmental changes, but the averages of the percentages of infertile eggs in the groups which

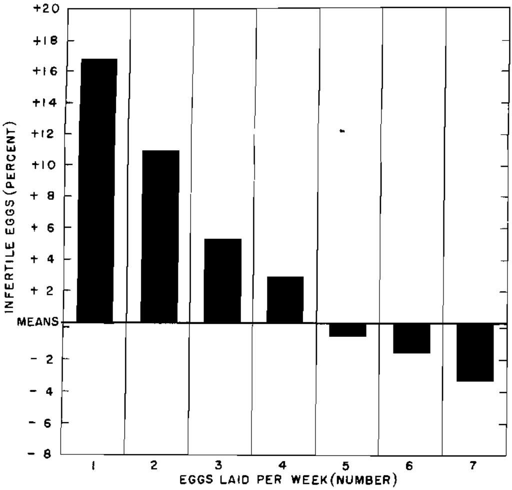

FIGURE 1.—The proportion of infertile eggs in groups of eggs which were laid at rates of from one to seven per week. Each column represents the average of the deviations from the mean for each of the 5 years (see text). The total number of eggs included in each group is shown in table 2.

were laid at different rates show a striking and consistent decline as the rate of egg production increases. In the group of eggs laid at the rate of only one per week, the proportion of infertiles (31.2 percent) is nearly three times as high as in the eggs laid at the rate of seven per week, where it was only 11.8 percent.

Because the percentages of infertility in the different years were not uniform, it is questionable whether the data for all 5 years should be combined. To overcome this difficulty and to permit comparisons of infertility in the groups of eggs laid at different rates the following procedure was adopted. To remove the differences between years the percentage infertile (weighted average) among all eggs for any 1 year

was calculated and used as a base line. This percentage was then subtracted from the percentage of infertiles that year in each of the seven groups of eggs which were laid at rates of one to seven, respectively, per week. This was done with the data for each of the 5 years. Finally, the plus and minus differences thus obtained for corresponding groups (one to seven eggs) during each of the 5 years were averaged (unweighted) to show the average deviation in percent from the five means in the proportion of infertle eggs in each of the seven groups (fig. 1).

This combination of the 5 years' data shows that the proportion of infertiles was less, by 19 percent of the total eggs, among those laid at the rate of seven a week than among those laid at the rate of one a week. Since among all eggs studied less than 15 percent were infertile (table 2) the columns in figure 1 further indicate that the proportion of infertiles among eggs laid at the rate of one a week was more than twice that found among eggs laid at the rate of five to seven a week.

It must be remembered that nearly every hen is represented in two or more of the groups in figure 1 and that here the influence of intensity of production upon fertility is more accurately measured than in the longer periods considered in table 1, where all eggs from a given hen were grouped together regardless of changes in the intensity of production. It is important to note that in this phase of the study greater extremes in the intensity of production (14 to 100 percent) were measured than are analyzed in table 1. Correspondingly greater differences in fertility are also shown, and when one considers the many uncontrolled factors contributing to infertility it is a noteworthy fact that the average infertility consistently declines with each increase of only one egg per week in the rate of production.

Number of Eggs per Clutch

The number of eggs per clutch, although an excellent measure of intensity, is more susceptible to error in an experiment of this type than is the number of eggs per week. If a hen lays four eggs per week and one is lost or broken, the week's eggs would be entered in the adjoining column. If, however, one egg is lost from a clutch of four, it may create two apparent clutches which are removed one to three groups from the correct one. Obviously, the larger the clutch the greater the chance of such error occurring, and the more serious the displacement when it does occur. Nevertheless, the large number of eggs considered in this study should make apparent any marked relationship that may exist between size of clutch and degree of infertility. In considering the results of this study it is important to remember that errors in the data will tend to minimize rather than to magnify the apparent degree of such a relationship.

Accordingly the incubated eggs of 1,178 hens were tabulated with respect to the size of the clutch in which they were laid, and the proportions of infertiles in clutches of different sizes were calculated. All complete clutches laid during February, March, and April were included, with the result that larger numbers of eggs and somewhat different levels of infertility are recorded in table 3 than in table 2. As with the two measures of intensity previously considered, the size

of clutch was also found to bear a distinct relationship to the proportion of the eggs laid which were infertile. The clutches which were small, indicating a low intensity of production, included a greater proportion of infertiles than larger clutches, the eggs of which were laid at a more rapid rate.

TABLE 3.—*The proportion of infertile eggs among those laid in clutches of one to three and of more than three, 1935–39*

Year	Hens	Eggs laid	Infertile eggs in clutches of—		Difference	$P(\chi^2)$
			1 to 3 eggs	4 to 41 eggs		
	Number	*Number*	*Percent*	*Percent*		
1935	217	8,739	18.3	12.1	6.2	∠0.001
1936	237	7,762	29.4	22.7	6.7	∠.001
1937	245	7,914	13.3	10.6	2.7	∠.001
1938	249	6,745	13.3	10.0	3.3	∠.001
1939	230	9,192	12.1	10.6	1.5	∠.05
Total	1,178	40,352				

The data for the different years are not sufficiently homogeneous to be combined for the calculation of statistical significance of differences between the eggs in clutches of different size. Accordingly, for each year the infertility of eggs laid in clutches of one to three (inclusive) was compared with the infertility of the remaining eggs (table 3). Although the number of eggs considered in each comparison is thus reduced to less than 10,000, these numbers were adequate to show that in each year 1.5 to 6.7 percent fewer of the total eggs were fertile in the small clutches than in the large. The proportion of infertile eggs was accordingly 14.6 to 29.6 percent greater in the small clutches than in clutches of four or more eggs. Although these differences are less striking than those presented in figure 1, they are nevertheless shown by the χ^2 test to be highly significant. With odds of more than 999:1 against the occurrence by chance of the differences found in 4 of the 5 years, it is obvious that eggs from small clutches tend to be less fertile than those from large clutches.

DISCUSSION

A study of infertility in the fowl is simplified, as compared with mammals, by the fact that a comparatively accurate distinction can be made between infertility and early embryonic mortality. That it is nevertheless difficult to ascribe to any particular cause a specific degree of infertility is shown by the diverse reports that age (*17, 23*), temperature (*14, 26*), breed (*16*), size (*29*), disease (*12*), absence of genital papilla in males (*9*), preferential mating (*28, 30*), number of matings (*8*), selective fertilization (*1, 7, 10*), and the time elapsed after the last mating (*7, 8, 18, 19*) may all influence the percentage of infertile eggs produced.

It is therefore desirable whenever possible to use more than one measure of a factor that is thought to influence degree of infertility. It has been pointed out that for each of the three measures of intensity used in this study the inherent errors of the data or the methods of analysis may modify the results. This is well illustrated by the

disagreement among previous reports. Chlebaroff (*5*) expressed the opinion that the more intense the laving activity, the larger is the proportion of fertilized eggs laid, and vice versa. No data were given to substantiate this statement. Nicolaides (*25*) reported that of 26 females laying an average of 17.7 eggs during an experimental period, the mean percentage of fertile eggs was 79.2 and 84.2, respectively, for those laying fewer or more eggs than the average.

It was reported by Montemayor (*24*) that there is no definite relation between production and fertility. His conclusion is based upon the incubation of 18,669 eggs during 280 hatches distributed through a 48-month period (1928–31). Eggs from three different breeds were incubated in 10 different incubators. Although he agrees with the observations of others that fertility is a matter of individuality, nevertheless he selected his eggs for incubation from the daily production of the flocks studied and calculated percentage production from the flock average. It is a well-recognized fact that fluctuations in flock production are frequently merely a reflection of the proportion of birds which are laying at a given time, and not necessarily any indication of the individual's rate of production. Such individual differences as existed could not be measured by this method of selecting eggs and therefore any relationship within the individual between rate of production and infertility could not be detected.

After the preceding analyses (tables 1–3) were completed, Funk (*11*) reported a study in which one of the three measures of intensity used here, size of clutch, was shown to be associated with degree of infertility. With methods much superior to those of Montemayor (*24*) he found that eggs from clutches of two to six eggs each included 5.2 to 12.6 percent fewer infertiles than those laid in clutches of one egg each.

The use in the present study of data obtained over a 5-year period from large numbers of both males and females has rendered inconsequential such errors as are attributable to differences in the environment and genotype of the birds. Inaccuracies arising from errors in trap-nesting, recording, and handling of the eggs are largely distributed by chance in such large populations as those studied. The danger of drawing false conclusions from results obtained by a method which is handicapped by error in sampling has been minimized by comparing the results obtained by three different methods of measuring intensity. It is therefore highly significant that with each of the three methods of analysis a markedly greater proportion of infertiles was found among those eggs which were produced during periods when the intensity of production was relatively low.

The conclusion seems justified therefore that insofar as this relationship is concerned those details of poultry management which tend to induce maximum intensity of production during the incubation season will also tend to induce maximum fertility.

STUDIES TO DETERMINE THE CAUSES OF INFERTILITY

The mere fact that fluctuations in the rate of egg production are associated with measurable differences in fertility does not explain the reasons for this relationship. Since the degree of infertility obviously cannot influence the rate of ovulation, it appears most logical that rate of ovulation directly affects the fertility of the eggs laid. In

searching for some basis for this relationship it was therefore suspected that the means by which intensity of production may affect fertility is most likely to be some function of the ovary which varies directly with rate of ovulation.

Two facts make it appear that different rates of ovulation are associated with the secretion of different amounts of hormone by the ovary. (1) It has been shown by Corner (*6*) that the probable site of secretion of estrogen is the theca interna of the Graafian follicle. Marlow and Richert (*21*) have shown that the follicular membranes of fowl contain more than four times as much estrogen per unit of weight as the whole ovary with ova less than 6 mm. in diameter. (2) The observations of Warren and Conrad (*31*) show that the ova of the good layer develop at the same rate as those of the slow layer, but that the rapid layer has at any one time more ova in the final stages of growth. Thus the hen which ovulates at a rapid rate has a larger area of follicular (estrogen-secreting) tissue present in the body than has the hen which ovulates less frequently. That the volume of theca interna is also greater in the hen with many large follicles than in the hen with few is obvious from the fact that in the follicle of a very young oöcyte there is no theca interna (*27*).

High levels of estrogenic hormone in the body might influence fertility in the fowl (1) by increasing the ability of the ova to be fertilized; (2) by prolonging the functional survival of sperm in the oviduct; or (3) by inducing greater sexual receptivity, or desire for copulation on the part of the female. All three of these possibilities were studied experimentally.

Susceptibility of the Ova to Fertilization

Susceptibility of the ova to fertilization is a characteristic which is not readily studied by direct methods. The incidence of scattered infertiles among the fertile eggs following a single insemination suggests that it may be more difficult to fertilize some ova than others. However, suitable methods of study might demonstrate that many *such "infertiles" are actually fertile,* the embryo having *died* at a very early stage of development.

With the data obtained for this study only one measure of the influence of estrogens upon the ability of ova to be fertilized is possible, and that is effective only if very rapid elimination of the hormone from the body occurs following secretion. According to the explanation given by Warren and Conrad (*31*), there should be a maximum number of large ova present in the ovary at the beginning of a clutch, and a minimum at the end of the clutch, or when the last ovulation of a clutch occurs. There might, therefore, be slightly less estrogen in the body when the last two ovulations in a clutch occur than at the time of the first two. With this in mind the fertility of the first two eggs was compared with the fertility of the last two eggs in clutches of four or more. Such an analysis shows a difference of less than 2 percent between the two groups for each of the 5 years studied (table 4). Since the differences do not consistently favor either group and are obviously of no significance, it is clear that the position of the egg in the clutch does not influence its capacity for fertilization.

TABLE 4.—*The proportion of infertile eggs among the first two and last two eggs in clutches of four or more, 1935–39*

Year	Pens	Hens	Clutches	Infertile eggs in	
				First 2 of clutch	Last 2 of clutch
	Number	*Number*	*Number*	*Percent*	*Percent*
1935	15	217	732	11.5	13.4
1936	17	237	797	23.0	23.2
1937	20	245	557	10.9	10.4
1938	18	249	580	10.0	9.7
1939	21	230	751	9.2	11.0
Average (unweighted)				12.9	13.5

FUNCTIONAL SURVIVAL OF SPERM IN THE OVIDUCT

It has been frequently demonstrated that the oviduct is conditioned for reproduction by estrogenic hormone secreted by the ovary. Ovariectomy prevents this conditioning process. When a laying hen ceases to ovulate, marked atrophy of the oviduct occurs, indicating a marked reduction in the secretion of estrogens in the absence of large ovarian follicles. The oviduct again hypertrophies at the onset of ovulation or following the injection of synthetic estrogen. Such conditioning may conserve the energy and prolong the life of the sperm held in the oviduct, thereby prolonging the period of fertility following an insemination.

To test this hypothesis two experiments were made. First, two similar groups of hens were artifically inseminated by the method of Burrows and Quinn (*4*) and the duration of fertility determined. Each female was inseminated with 0.1 cc. of the mixed semen obtained from several males. This was twice the amount reported by Burrows and Quinn (*3*) as giving normal fertility, but it was used in this experiment to avoid fluctuations such as might be expected from marginal dosage. The hens of one group were given supplementary estrogen [3] to ascertain its influence upon sperm survival. Since the hormone might be expected to increase the fertility of hens that lay at a slow rate to the approximate level of fertility of hens that lay at a rapid rate, only those hens are compared in table 5 which laid at a rate of less than 55 percent (of the assumed maximum of one egg daily) or more than 70 percent. These levels of selection were used because they most nearly divide the populations of the larger groups into thirds. The fact that noninjected controls showed slightly longer duration of fertility (table 5, battery 2) than those receiving the supplementary hormone suggests that estrogen does not prolong the life of sperm in the oviduct.

Because of the small number of hens which laid at a slow rate, a second study (also reported in table 5) was made by comparing the duration of fertility of those hens which lay at a relatively slow rate, and therefore presumably secrete less hormone, with hens which ovulate at a rapid rate. Hens in pen F–61 (not in cages) were employed for two tests. It is of interest to note that each of the four groups (table 5) which laid at a rate of less then 55 percent laid fertile

[3] The hormone used was Progynon-B (estradiol benzoate) kindly furnished by Dr. Max Gilbert, of the Schering Corporation, Bloomfield, N. J.

eggs for a shorter average time than the groups of hens which laid at a rapid rate. Because part of the hens kept in cages were injected with hormone and also because the group as a whole included relatively few hens which ovulated at a slow rate, they should not be used in a statistical test for the significance of correlation between the rate of ovulation and the percentage of eggs laid which were infertile. This reduces the number of individuals available, but for the 52-hen tests remaining (table 5, F–61) there exists a correlation coefficient of 0.29 ± 0.13 between rate of egg production and duration of fertility in days. Inasmuch as there is a probability of less than 5 percent that such a relation may have resulted by chance, it may be concluded that sperm tend to survive somewhat longer in the oviducts of rapid than of slow layers.

TABLE 5.—*Duration of fertility following artificial insemination of hens that laid at a slow or at a rapid rate, as affected by supplementary estrogen*

Group or pen	Hens	Production [1]	Hens with production > 55 percent		Hens with production < 70 percent	
			Number	Duration of fertility	Number	Duration of fertility
	Number	*Percent*		*Days*		*Days*
Battery 1 injected [2]	16	65.4	2	8.5	10	10.7
Battery 2, controls	23	66.6	5	8.8	13	12.2
F–61, first test	29	63.6	10	10.5	12	14.3
F–61, second test	23	58.8	6	9.7	7	10.3
Average (unweighted)	-----	----------	---	9.4	------	11.9

[1] $\frac{\text{Number of eggs} \times 100}{\text{Number of days} \times \text{hens}}$

[2] Injected with 100 rate units Progynon-B daily beginning 2 days before insemination.

INDUCTION OF SEXUAL RECEPTIVITY

Observations in this laboratory and the published results of others show that in some hens fertility declines rapidly following insemination. With such individuals, relatively frequent mating is obviously necessary if the highest possible proportion of the eggs laid are to be fertile. That a high degree of infertility may be due to a lack of sexual receptivity on the part of the female is indicated by the following experiment.

During the 1939 incubation season there were 11 hens in the breeding pens at Cornell University which were laying few, if any, fertile eggs. These hens were therefore inseminated artificially with semen from the respective males with which they had been mated. The proportions of fertile eggs among those laid during the 10 days previous to artificial insemination, and from the second to the eleventh day after the first artificial insemination, were as follows:

Before artificial insemination 80 eggs, 2.5 percent of which were fertile.

After artificial insemination 138 eggs, 60.9 percent of which were fertile.

The fact that the hens in this experiment laid fertile eggs following insemination strongly suggests that the previous infertility was a result of failure to copulate. These data do not show that the female

was wholly responsible, but since it has previously been shown that matings are largely controlled by the female (*22*, *28*), it is apparent that desire for copulation on the part of the female has a marked influence upon the fertility of the eggs laid.

It is generally believed that nonlaying hens mate infrequently, if at all. This, in addition to the above results, suggests two questions concerning the method by which frequency of ovulation can influence infertility. (1) Are hens which lay most rapidly more fertile because they copulate most frequently; (2) Can the frequency of copulation be increased in the laying and nonlaying hen by injections of synthetic estrogen?

Data have been obtained by two authors which furnish a specific answer to the first of these questions. By rearranging the data of Wilkins [4] and Heuser [5] the summary in table 6 was obtained to show the average number of copulations made by hens which do not ovulate, or which ovulate at a moderate or a rapid rate.

Expressed in a different way, Heuser found a coefficient of correlation of 0.609 ± 0.109 between the number of matings and the rate of egg production. These observations make it very clear that those hens which ovulate most rapidly also copulate most frequently.

TABLE 6.—*Rearrangement of data from Wilkins and from Heuser (see text) to compare number of eggs laid with the average number of copulations made by hens that ovulated rapidly, moderately rapidly, or not at all.*

Investigator and length of experimental period	*Hens*	Eggs laid (average)	Copulations (average)
	Number	*Number*	*Number*
Wilkins, 18 days	11	0.0	4.1
	16	6.2	13.3
	13	13.6	22.8
Heuser, 13 days	4	.0	2.0
	15	4.2	14.9
	17	9.4	21.8

To study the second question, concerning the influence of synthetic estrogen upon receptivity of the female, an experimental pen was assembled which included three immature pullets 15 weeks of age, three nearly mature but nonlaying pullets, four pullets which had recently started to lay, four yearling hens which were laying at a rapid rate, and three hens from a strain bred for low fecundity (*13*) which were genetically incapable of ovulating during September under normal conditions.

Males were placed with these birds during observation periods of 4 to 5 hours per day and the mating reactions recorded on September 17, 19, 20, 21, 22, 25, 27, 29, 30, and October 1. Following observations on September 20, and daily thereafter for 11 days, some of the females were injected (intramuscularly) with 100 to 1,000 rat units of Progynon-B (estradiol benzoate).

The daily dosage of 1,000 rat units of estrogen was considered quite large for a hen, since the injection of 900 to 1,500 rat units during 30 hours will regularly induce oestrus in the ewe (*20*). However, with

[4] WILKINS, R. H. SOME FACTORS INFLUENCING THE FERTILITY AND HATCHING POWER OF EGGS OF THE DOMESTIC FOWL. (Thesis, Cornell Univ.) 1915.

[5] HEUSER, G. F. A STUDY OF THE MATING BEHAVIOR OF THE DOMESTIC FOWL. (Thesis, Cornell Univ.) 1916.

one exception there appeared to be no relationship between the amount of hormone injected and frequency of copulation among the laying hens. The 15-week-old pullets showed no desire to copulate at any time and the largest number of copulations made in the 11-day period by any of the nonlaying yearling hens was only three.

It is not probable that this apparent inability of estrogen to induce copulation was a result of insufficient dosage. In those hens which were not laying there was a marked hypertrophy of the oviduct following injections of the hormone (fig. 2). Although the resting oviduct attained a weight during this 12-day experiment of only about one-half that of the oviduct in a laying hen, certainly such marked stimulation given to hens already moderately stimulated by an active ovary should affect frequency of copulation if estrogens were the primary cause of sexual receptivity.

Discussion

The fact that no significant difference in fertility was found between the first two and last two eggs in a clutch (table 4) substantiates the recent conclusion by Funk (*11*) that "The position of the egg within a given clutch apparently is not related to fertility."

The consistently longer duration of fertility in the hens which ovulated most rapidly (table 5) and the significant coefficient of correlation between rates of ovulation and duration of fertility in the hens of F-61 show that spermatozoa probably survive longer, on the average, in those hens which ovulate rapidly than in those which lay at a slow rate. In a similar experiment reported by Warren and Kilpatrick (*32*) hens which laid at a rate of 60 percent or more had an average duration of fertility 1.64 days longer than that of hens laying at a rate of less than 60 percent, but this difference was not considered significant.

The idea may be inferred from much of the literature concerning sexual behavior in the fowl that because copulation occurs so frequently and the average duration of fertility may be more than 1 week, the frequency of copulation is not a limiting factor in the practical attainment of maximum fertility. That it is actually of considerable importance is shown, however, by the improved fertility obtained in the writer's experiments following artificial insemination of hens which were quite infertile when mated naturally with the same males. The value of frequent matings was also shown by Curtis (*8*), who found that although more than one mating did not lengthen the duration of fertility, it did increase the proportion of the eggs laid which were fertile from 72.6 to 83.3 percent. The additional fact that the spermatozoa of a new male quickly supplant those of a preceding male (*7*, *32*), is indirect evidence that sperm, though long-lived, retain maximum efficiency for a relatively short time.

One limiting factor affecting the average fertility in a flock is, not the average frequency of copulation by all birds, but rather the minimum number of copulations by the few birds that pull down the flock average. The observations of Wilkins [6] show that it is not uncommon for some laying hens to mate only once in two or more days. Since some natural matings are infertile (*22*, *25*) the actual time between effective matings is longer than observations indicate.

[6] See footnote 4.

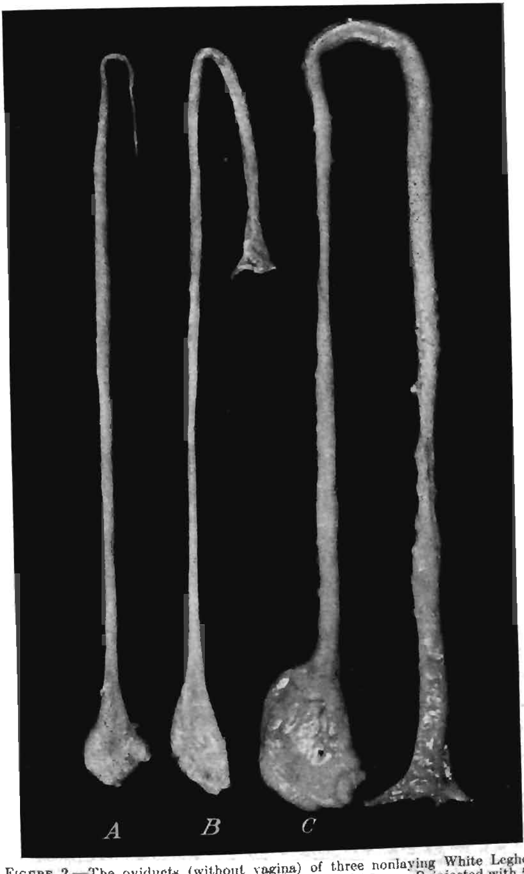

FIGURE 2.—The oviducts (without vagina) of three nonlaying White Leghorn yearling hens: *A*, Control, weight 3.6 gm., length 25.5 cm.; *B*, injected with 500 rat units of Progynon-B daily for 12 days, weight 4.4 gm., length 31.5 cm.; *C*, injected with 1,000 rat units of Progynon-B daily for 12 days, weight 20.2 gm., length 53.0 cm.

Another limitation upon fertility of the flock results from the inability of some females to maintain fertility more than 2 or 3 days after a single mating. Curtis (*8*) found the duration of fertility following copulation to be as short a time as 2 days. Kaupp (*18*) reported a decline of fertility after 3 days following removal of the male. Bonnier and Trulsson (*2*) found that on the second day after artificial insemination a larger proportion of fertile eggs was obtained than on subsequent days. Similar results were obtained with two groups of hens in this study (F–61, table 5). The hens of one group were artificially inseminated once and gave higher fertility (100 percent) on the second day following than they gave later. Hens of the other group were inseminated on each of 2 consecutive days. Eggs laid the third day after the first insemination were lost, but the records for other days show that fertility declined after reaching a maximum of 94.7 percent on the second day.

Among the hens which copulate frequently the duration of fertility following mating can have relatively little influence upon the proportion of the eggs laid which are infertile. The results of this study indicate that the principal reason for the high level of infertility among eggs which are produced at a slow rate is the tendency for hens to copulate infrequently during periods of infrequent ovulation. Just how sexual receptivity is controlled by the rate of ovulation, or by those factors which influence rate of ovulation, remains to be determined.

SUMMARY

An analysis of the incubation records of more than 1,200 hens was made to determine the relationship between the proportion of eggs laid which are infertile and the rate of egg production.

The hens which laid 13 to 22 eggs during a 6-week period produced a larger proportion of infertile eggs during that time than birds which laid at a more rapid rate.

The eggs laid during weeks when one to seven eggs were laid showed, respectively, a degree of infertility which was inversely proportional to the number of eggs laid per week.

The proportion of infertile eggs was significantly higher among those which were laid in clutches of one to three eggs than in those laid in clutches of more than three.

During periods when the rate of ovulation is low, hens copulate less frequently and probably have a shorter duration of fertility following insemination. These two factors, particularly the first, are believed to be responsible for the high level of infertility among eggs laid during periods when the intensity of production is low.

The duration of fertility following artificial insemination was not prolonged by daily injections of 100 rat units of Progynon–B per bird.

Sexual receptivity in the fowl was influenced only slightly, if at all, by 12 daily injections of 100 to 1,000 rat units of Progynon–B.

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THE CYSTINE CONTENT OF ELEVEN VARIETIES OF SOYBEANS [1]

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INTRODUCTION

The soybean (*Soja max* (L.) Piper) is fast becoming a factor of major importance in American agriculture. A valuable crop to the agronomist, a source of raw materials for many industrial commodities, such as glue, plastics, paint, artificial wood, etc., as well as a valuable feed and food supplement for both man and animal, the soybean occupies an important place among the cultivated crops in many parts of the world. While soybean hay, whole soybeans, and soybean oil are valuable foodstuffs, the proteins of the beans are of chief interest and importance so far as the nutritive value of the crop is concerned.

By 1925, 1,133 varieties had been described, and of these more than 100 named varieties are widely grown or are being distributed in the United States (*16*).[2] Some varieties are useful for their oil content or for their feeding value for animals. However, a number of the newer varieties are valuable as food for human beings. Woodruff (*28*) of the University of Illinois has tested 467 varieties of edible soybeans for their eating qualities and has rated 6 as very good and 70 as good.

The value of soybeans or soybean oil meal, or of any feed, as a source of protein to the animal body depends not only upon the total amount of protein present, but also upon its digestibilty and biological value when consumed. That the total amount of protein in a sample of soybeans may vary widely, depending upon the variety, soil fertility, climatic conditions, etc., has been shown by a number of investigations. References to investigations of this nature to 1938 may be found in a review by the Soybean Nutritional Research Council (*10*).

Osborne and Campbell (*19*) isolated several types of proteins from soybeans and gave to the principal one, a globulin, the name glycinin. This protein amounted to 16.6 percent of the meal or between 80 and 90 percent of the total protein present. They also obtained evidence of the existence of another globulin which was even more soluble than glycinin. In addition to these two globulins there were isolated a legumelin, amounting to about 1.5 percent of the seed, and a small amount of proteose.

A number of investigations (*9, 12, 14, 15*) have indicated that the proteins of raw (usually defatted) soybeans have a low nutritive value. Mitchell and Smuts (*15*) and, later, Shrewsbury and Bratzler (*22*), Hayward, Steenbock, and Bohstedt (*8*), and Smuts and Marais (*23*) have shown that the amino acid limiting the utilization of the absorbed nitrogen from soybeans was cystine.

[1] Received for publication April 15, 1940.
[2] Italic numbers in parentheses refer to Literature Cited, p. 212.

In view of these findings and the wide variations in the protein content of different varieties of soybeans, it is of interest to examine, in more detail, the chemical nature and, in particular, the cystine content of different varieties of soybeans. Unfortunately there has been but little work done on the methionine content of these beans.

Osborne and Clapp (*20*) analyzed glycinin and found the amino acid content to be similar to that of casein. They reported, however, no cystine values for the isolated protein. Csonka and Jones (*4*) also analyzed the glycinin from the seeds of several varieties of soybeans for cystine, tryptophane, and tyrosine. These workers reported a variation in the cystine content between 0.74 percent for the Illini variety and 1.45 percent for the Manchu variety. Csonka, Murphy, and Jones (*3*) presented evidence which indicated that glycinin, as ordinarily prepared, was not an individual protein and Csonka and Jones explained the wide variation in the cystine content of their different glycinin preparations on the basis of variations in the relative proportions in which the different globulins comprising the glycinin fractions were present in the different varieties of soybeans.

Later (*5*), Csonka and Jones, after failing to obtain satisfactory cystine determinations on defatted soybean meal, determined the cystine content of 10-percent sodium chloride extracts (containing 85 to 90 percent of the total nitrogen of the meal) of six varieties of soybeans. At the same time tryptophane and tyrosine were determined directly on the defatted meals of the same varieties. Using a modification (*2*) of the Sullivan (*24*) method, they found variations in the cystine content of the defatted meals of from 0.287 percent for the Illini variety to 0.491 percent for a Herman variety.

Sasaki (*21*) analyzed soybean protein for several amino acids but reported no values for either of the sulfur-containing amino acids. Mashino and Nishimura (*13*) studied the nitrogen distribution, by the Van Slyke method, in the defatted beans from two varieties, Tsurunoko-daizu and Machurian, the latter of which was analyzed in the unheated and heated states after the oil had been removed either with solvent or by pressure. They found but little variation in the nitrogen distribution of their samples and reported an average of 1.74 percent of cystine nitrogen. Earlier, and also using the Van Slyke method, Hamilton, Uyei, Baker, and Grindley (*7*) reported an average value of 1.46 percent in Medium Early Yellow soybeans, and Nollau (*17*) reported 1.52 percent, both values being cystine nitrogen expressed as a percent of the total nitrogen.

Baernstein (*1*) found, in his study of a number of proteins, that soybean glycinin contained 1.84 percent methionine. The methionine sulfur amounted to 47.2 percent of the total sulfur. However, Tomiyama and Hanada (*25*) were able to isolate but 0.08 percent of methionine from dry, ash-free soybean protein.

The study to be reported here is concerned with the cystine content of 11 varieties of soybeans, all grown in the same year (1933) on a small plot of ground, the soil of which was very uniform. The beans were taken from groups of plants systematically replicated 10 times over the entire plot.[3]

[3] These samples were furnished by Prof. C. M. Woodworth of the Department of Agronomy.

PROCEDURE

Each of the 11 varieties of soybeans used was clean and well cured when received. The beans were first ground, avoiding the generation of heat, so as to pass through a 40-mesh sieve. In a few cases the beans were ground even finer. The method of preparation of a fat-free, carbohydrate-free protein sample was similar to that used by Hamilton, Nevens, and Grindley (*6*), with only slight modifications. In this procedure triplicate 15-gm. samples of the finely ground, dry beans were thoroughly extracted, first with absolute ethyl ether, then with absolute ethyl alcohol. These extracts were discarded after they were found to be practically free of nitrogen.

The residues from the ether and alcohol extractions were then extracted with dilute sodium hydroxide solutions. Eight to ten extractions, using 400 cc. of 0.1-percent Sodium hydroxide for each extraction, were first made. Then extractions were made in which 200 cc. of 1-percent sodium hydroxide was used for each extraction. These extractions were continued until the extract no longer gave a test for protein, usually 6 to 8 extractions being necessary. Centrifuging and decanting were used to separate extract from residue. Each extract was immediately neutralized with acetic acid after being decanted.

The combined extracts from each sample were then carefully concentrated to about 200 cc. An equal volume of concentrated hydrochloric acid was added and the protein hydrolyzed by gentle boiling under a reflux condenser for about 20 hours. The hydrolyzate was then cautiously concentrated in a beaker to about 125 to 150 cc. and neutralized with 40-percent potassium hydroxide solution. After the solution was made barely acid to litmus, 2 to 3 gm. of norit were added, and the mixture boiled gently for a few minutes. The norit was filtered off and washed with hot water. After the filtrate had stood overnight, it was again treated with a small amount of norit. The solution thus obtained was clear and slightly yellowish, but this color did not seem to interfere with the colorimetric estimation of cystine.

Cystine was determined in the extracts by Lugg's (*11*) modification of the Sullivan (*24*) method.

RESULTS

The results of this study are presented in the accompanying tables. Table 1 gives the usual chemical analyses and table 2 gives the results of the cystine determinations on the 11 varieties of soybeans. The routine chemical examination shows a considerable varietal variation between the samples, this being particularly true of the protein content. The percentage of protein (total nitrogen $\times$ 6.25) varied from 35.88 (Peking) to 43.69 (Manchu), averaging 39.11 for the 11 varieties. The ether extract varied from 16.07 (Ito San) to 19.16 (Mansoy), averaging 17.86 percent for all varieties. Since these varieties had about the same moisture content and since all were grown in the same year on uniform soil in a small plot of ground, the differences observed strongly indicate varietal differences of considerable magnitude.

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TABLE 1.—*Percentage composition of 11 varieties of soybeans grown in Illinois and harvested in 1933*

Variety	Moisture	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Total nitrogen
Illini	7.06	37.75	18.82	4.37	27.28	4.72	6.04
Ohio 13-177	7.04	38.31	18.75	4.54	26.32	5.04	6.13
Harbinsoy	7.12	39.69	18.45	4.24	25.50	5.00	6.35
Virginia	6.89	38.81	17.90	4.65	26.71	5.04	6.21
Mansoy	7.17	36.25	19.16	4.41	27.84	5.17	5.80
Ito San	6.68	41.44	16.07	3.97	26.74	5.10	6.63
Dunfield	6.73	36.38	17.62	4.12	30.45	4.70	5.82
Manchu	7.34	43.69	17.80	3.69	22.44	5.04	6.99
Peking	7.20	35.88	17.38	5.03	29.41	5.10	5.74
Mandarin	7.82	41.44	16.66	4.12	24.92	5.04	6.63
Morse	7.41	40.56	17.91	3.97	25.02	5.13	6.49
Average	7.13	39.11	17.86	4.28	26.61	5.01	6.26

TABLE 2.—*Cystine content of 11 varieties of soybeans*

Variety	Total nitrogen extracted	Cystine content		
		In whole soybeans [1]	Cystine nitrogen as percent of total nitrogen	Cystine per gram of nitrogen
	Percent	*Percent*	*Percent*	*Milligrams*
Illini	96.6	0.366	0.696	59.7
Ohio 13-177	99.1	.266	.433	42.3
Harbinsoy [2]	92.8	.256	.464	39.8
Virginia	96.5	.213	.385	33.1
Mansoy	95.5	.553	1.042	89.4
Ito San	90.8	.413	.703	60.3
Dunfield	97.9	.439	.858	73.7
Manchu	99.4	.451	.742	63.7
Peking	95.6	.276	.564	48.4
Mandarin [2]	94.4	.507	.848	72.8
Morse	99.2	.346	.617	53.0
Average	96.2	.371	.668	57.8

[1] The coefficients of variability for duplicate or more determinations on the same variety (the number of separate determinations made on the same variety are indicated by the numbers in parentheses) were as follows: Illini (3) 2.5 percent, Ohio (3) 2.6, Harbinsoy (5) 6.2, Virginia (2) 3.7, Mansoy (3) 0.9, Ito San (3) 3.4 Dunfield (3) 5.7, Manchu (3) 2.7, Peking (3) 5.4, Mandarin (2) 5.1, and Morse (2) 0.86.

[2] The extraction procedure in this case was modified by using a 2-percent trichloracetic acid extraction after the alcohol extraction and then precipitating the protein extracted with colloidal iron.

It may be noted from table 2 that the cystine determinations were made on extracts which contained on an average 96.2 percent of the total nitrogen in the whole beans. In only 2 of the 11 samples was there less than 93 percent of the total nitrogen in the extract.

Regardless of the manner in which the cystine is expressed, i. e., as a percent of the whole seed, as cystine nitrogen in percent of the total nitrogen, or as milligrams of cystine per gram of total nitrogen, there are large and significant differences. The percentage of cystine in the whole seed varied from 0.213 (Virginia) to 0.553 (Mansoy). The percentage of the total nitrogen which was cystine nitrogen varied from 0.385 (Virginia) to 1.042 (Mansoy), that of Mansoy being nearly 200 percent greater than that of Virginia. Expressed as milligrams of cystine per gram of total nitrogen, the variation was 33.1 (Virginia) to 89.4 (Mansoy).

Because of the completeness with which the total nitrogen of the beans was extracted, the cystine values reported here should repre-

sent more accurately than any heretofore published the actual amount of this amino acid present in the whole bean. The only previously reported results with which the results of this investigation may be compared, are those reported by Csonka and Jones (*5*), and even here the samples were not exactly comparable since Csonka and Jones analyzed a 10-percent sodium chloride extract (containing 85 to 90 percent of the total nitrogen) of the defatted meals. And of course difference in soils and environment might also add to any differences observed. Nevertheless, there were two varieties studied by both laboratories. Csonka and Jones reported 0.287 percent of cystine in defatted meal from Illini beans, while 0.451 percent of cystine may be calculated to have been present in the fat-free beans analyzed by the writers. Similarly, Csonka and Jones found 0.388 percent and the writers found 0.334 percent of cystine in the fat-free Peking variety. That there is probably a higher content of cystine in the glycinin fraction than there is in the entire mixed proteins of the soybean, is indicated by Csonka and Jones (*4*), who reported 0.74 and 1.45 percent cystine in the glycinin from the Illini and Manchu varieties, respectively, while the writers found but 0.366 and 0.451 percent of cystine in the whole beans of the same varieties.

While the results of the present study, as well as those of previous investigations, strongly indicate considerable varietal differences with respect to the cystine content of soybeans, the effects of other factors should not be overlooked. The same varieties grown in a single location may also vary considerably from year to year. O'Kelly and Gieger (*18*) found, over a 7-year period, variations in the protein content between 35.55 and 40.67 percent for the Loredo beans and between 39.91 and 44.64 percent for the Mammoth Yellow variety. That other environmental conditions, such as rainfall, fertility of soil, and temperature, may cause large differences in the protein content of the same variety has been shown by Webster and Kiltz (*26*). These investigators reported the protein content of four varieties of soybeans grown in two different counties of Oklahoma. Considerable variation was found in all varieties, but by far the greatest was that exhibited by the Loredo beans that contained 35.00 percent of protein when grown in Craig County and 47.50 percent when grown in Payne County (at Stillwater).

Thus, while wide differences observed in this study in the cystine content of different varieties of soybeans probably represent true varietal differences, the above-mentioned studies, indicating that environmental factors may cause wide differences in the protein content of the same variety, suggest the probability that the small differences observed in the cystine content of different varieties may not be true varietal differences.

Since the mixed proteins, rather than isolated proteins, are used most often as human and animal foods, these values on the cystine content of several common varieties of soybeans should be of interest. Also since cystine has been shown to be the limiting amino acid in the utilization of certain soybeans, a knowledge of the varietal variation should be of value to all interested in using soybeans as a protein supplement. It is conceivable, on the basis of their cystine content, that certain varieties would find their greatest value for feeding purposes while other varieties may be grown for industrial

purposes. For those varieties in which cystine has been shown to be the limiting factor in the utilization of their protein, it is obvious (*27*) that methionine also must be present in insufficient amounts to satisfy the combined requirements for both sulfur-containing amino acids.

Although Baernstein (*1*) reported the presence of 1.84 percent of methionine in glycinin from soybeans, little is known concerning the variation of this amino acid in different varieties of whole soybeans. It is, however, reasonable to suppose that there exists among different varieties a variation of considerable magnitude in the methionine content.

The inadequacy of a chemical estimation of a food nutrient in satisfying the animal's requirements may be emphasized in this connection. Compared with other foods and feeds, soybeans in general are not particularly low in their content of sulfur-containing amino acids, as indicated by chemical analyses. Nevertheless the sulfur-containing amino acids limit the utilization of raw soybean protein in growing animals. Heating the soybeans apparently makes the cystine or its equivalent methionine more available so that the nutritive value of the heated protein is approximately equal to that of the raw protein supplemented with cystine (*8*).

SUMMARY AND CONCLUSIONS

The cystine content of extracts, containing on an average 96.2 percent of the total nitrogen content of 11 varieties of soybeans all grown in the same year on uniform soil, was determined. Expressed as a percent of the whole soybean seed, the cystine content varied from 0.213 for Virginia beans to 0.553 for Mansoy beans. Expressed in milligrams of cystine per gram of nitrogen, a variation from 33.1 for the Virginia beans to 89.4 for the Mansoy variety was found. These latter figures show nearly 200 percent variation, much of which probably represents true varietal differences.

Since cystine or its equivalent in nutrition has been shown to be the limiting factor in the utilization of at least certain varieties of soybeans, and since there may be large differences in their cystine content, it is concluded that perhaps certain varieties should find their greatest value as protein supplements in human diets and in animal rations while others should find their greatest usefulness in the industries.

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SOIL-FERTILITY STANDARDS FOR GROWING NORTHERN HARDWOODS IN FOREST NURSERIES [1]

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INTRODUCTION

This paper is supplemental to a previous report dealing with soil-fertility standards for growing northern conifers in forest nurseries (*9*).[3] The general principles and technique therein outlined were followed in the present study. The fertility standards for growing northern hardwoods in forest nurseries were arrived at by analysis of soils from normally developed productive stands that included the following species: Yellow birch (*Betula lutea* Michx. f.), hard maple (*Acer saccharum* Marsh.), basswood (*Tilia americana* L.), American elm (*Ulmus americana* L.), and white ash (*Fraxinus americana* L.).

No attempt was made in the present study to establish fertility standards for the heavy-seeded species, such as oak, hickory, and walnut. During their first growing season such trees feed to a large extent on nutrients of the seed, and are less dependent upon the soil-fertility level.

DESCRIPTION OF AREAS SAMPLED

As a general rule, northern hardwoods occur in nature as associates of various forest types, namely, hemlock, yellow birch, maple; maple, basswood, elm; oak, maple, ash; oak, ash, hickory, and so forth. Depending on circumstances, the soil sampling was applied to the entire forest type or to local groups of the species studied.

The total number of sampled areas was limited to 200. However, great care was exercised in the selection of localities; stands affected by logging, pasturing, or fire, as well as those younger than 100 years, were rejected. Nearly 60 percent of all samples were collected from the mixed stands of hard maple, basswood, and elm. Most of these stands could be classified as true virgin forest. The soil samples for yellow birch standards, constituting approximately 25 percent of the total number, were taken largely from groups of this species interspersed in stands of hemlock. Difficulties were experienced in locating undisturbed growth of white ash, and most of the samples were secured from comparatively well preserved wood lots or park areas containing a considerable percentage of this species.

The sampling was rather uniformly distributed throughout the following region: Northeastern Minnesota, including the Chippewa National Forest, as far as the southern boundary of Superior National Forest; the entire area of noncalcareous drift of Wisconsin, including

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[2] Credit is due E. L. Stone and D. P. White who assisted in field work and laboratory analyses.

[3] Italic numbers in parentheses refer to Literature Cited, p. 221.

Chequamegon and Nicolet National Forests; and the northeastern portion of upper Michigan, particularly the Ottawa National Forest. A few areas were included from southeastern Minnesota, northeastern Iowa, southern Wisconsin, northern Illinois, and western Indiana.

The soils analyzed were for the most part of a loam or silt loam texture having approximately 40 percent of silt (0.05–0.005 mm.) and 25 percent of clay (less than 0.005 mm.) particles. The soils supporting yellow birch, as a rule, belonged to the true Podzol type with a more or less developed layer of raw humus. The rest of the hardwoods studied were located on moderately or slightly leached soils with incorporated humus of a mull nature.

CONTENT OF NUTRIENTS AND ASSOCIATED CONDITIONS IN SOILS SUPPORTING DIFFERENT HARDWOOD SPECIES

The results of analyses of soils supporting different hardwood species for pH value (*5*), base-exchange capacity (*2*), total nitrogen (*1*), available nitrogen (*3*, *4*), available phosphorus (*6*), available potassium (*8*), replaceable calcium, and replaceable magnesium (*2*) are reported in table 1.

TABLE 1.—*Reaction, base-exchange capacity, total nitrogen, and available nutrients in soils supporting different hardwood species* [1]

Species	Reaction (pH)	Base-exchange capacity per 100 gm	Total N	Approximate level of available N	Available P_2O_5 per acre	Available K_2O per acre	Replaceable per 100 gm. Ca	Replaceable per 100 gm. Mg
		Milliequivalent	*Percent*	*Pounds*	*Pounds*	*Pounds*	*Milliequivalent*	*Milliequivalent*
Yellow birch	5.31±.07	11.67±.6	0.165±.010	32	63.3± 8.5	167.1±10.9	5.2±.4	1.6±.2
Hard maple	5.79±.12	13.76±.9	.185±.012	41	136.7±12.4	242.3±17.5	8.3±.9	2.1±.3
Basswood	5.83±.09	14.54±.6	.197±.009	47	159.8±11.4	273.3±15.0	9.1±.7	2.5±.2
American elm	5.88±.11	15.02±.8	.211±.007	45	174.5±12.9	274.7±17.4	9.8±.7	2.3±.1
White ash	6.23±.07	15.83±.9	.217±.012	54	185.3±14.2	293.8±21.4	11.2±.8	2.9±.3

[1] Standard errors given.

The mean values and standard errors presented in table 1 express the fertility of the upper 8-inch layer of the soil profile. These figures were obtained by an analysis of soil samples collected from the separate soil horizons, such as litter, duff, humic layer, and leached layer. The thickness of each horizon, its volume weight, and its content of nutrients were considered in computing the average fertility of each 8-inch layer.

The amounts of available nitrogen, i. e., ammonia and nitrates combined, vary greatly with the season and weather conditions. Because of this, medians instead of arthmetic means are reported for the content of these constituents. Soils under yellow birch contained soluble nitrogen predominantly in the form of ammonia. Soils under white ash had about 90 percent of their available nitrogen in the form of nitrates. The rest of the hardwood soils showed but a slight excess of nitrates over ammonia, on the average.

The analyses show that the nitrogen-phosphoric acid-potash ratio for yellow birch approaches 1–2–5, thus being the same as that

found for coniferous species. The ratio for the other hardwoods lies in the proximity of 1–3–5.

TABLE 2.—*Standards of fertility for nursery blocks raising different species of trees*

Species	Reaction (pH)	Base-exchange capacity per 100 gm.	Total N	Available P_2O_5 per acre	Available K_2O per acre	Replaceable Ca per acre	Replaceable Mg per acre
		Milliequivalent	*Percent*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Yellow birch	5.3	12.0	0.16	60	175	2,000	300
Hard maple Basswood American elm	5.8	14.0	.20	150	275	3,500	500
White ash	6.2	16.0	.22	185	300	4,500	700

Table 2 presents in round numbers the desirable levels of different soil factors which may serve as standards in the maintenance of nursery soil fertility. From a practical standpoint the soil-fertility levels for hard maple, basswood, and elm do not differ significantly. Therefore, average values given for these three species in table 2 seem to be well justified, especially if one considers the resulting simplification in nursery soil management.

In this study no attempt was made to correlate the analytical data with the results of greenhouse investigations. However, in order to provide a simple illustration of the workability of the standards proposed, a number of soils depleted in nutrients were placed in ½-gallon glazed jars, the fertility adjusted to the desired levels by the application of peat and mineral fertilizers, and the jars seeded to various hardwood species. Figure 1 shows the results achieved with American elm and white ash on a Miami loam soil which had been under cultivation for 70 years.

ADJUSTMENT OF SOIL CONDITIONS

The general directions for adjustment of fertility in nursery soils have been previously outlined (*9*). In growing hardwoods, however, special consideration must be given to the problem of nitrogen fertilization.

The majority of climax hardwood species are heavy nitrogen feeders. With a relatively high pH value of the soil, the activity of micro-organisms in hardwood beds is intensive. As a result, most of the available nitrogen occurs in the form of nitrate, i. e., an acid radical which cannot be held in soil by the base-exchange fraction. Nursery beds are subject to strong leaching because they are exposed both to natural precipitation and artificial irrigation. A combination of these factors may necessitate the addition of soluble nitrogen salts, such as ammonium sulfate, sodium nitrate, or ammonium nitrate, even on soils with adjusted fertility. These are usually applied as liquid fertilizer after nitrogen deficiency is manifested by discoloration of seedlings.

Table 3 gives the range of annual applications of elemental nitrogen likely to be required by different hardwood species. Under unfavor-

FIGURE 1.—Growth of hardwood seedlings on a depleted (*a*, *b*) Miami loam soil from the University of Wisconsin arboretum and on the same soil with the fertility adjusted (*c*, *d*) to the recommended standards: *A*, 4-month-old American elm; *B*, 5-month-old white ash.

able conditions, as, for example, in rainy summers or in seasons when warm periods alternate with heavy rains, nearly double the maximum amounts indicated in table 3 may be necessary to prevent nitrogen starvation of the stock.

TABLE 3.—*Approximate range of annual applications of elemental nitrogen or of nitrogen salts likely to be required by different tree species*

Form of nitrogen	Requirement per acre for—		
	Yellow birch	Hard maple, basswood, American elm	White ash
	Pounds	*Pounds*	*Pounds*
Elemental	20–40	30–50	40–60
20-percent ammonium sulfate	100–200	150–250	200–300
16-percent sodium nitrate		175–300	250–375

Yellow birch, as a rule, presents no problem in the selection of the kind of nitrogen fertilizer. In nature it feeds, by and large, on ammoniacal nitrogen, and it responds readily to ammonium sulfate. The rest of the hardwood species, especially white ash, under natural conditions feed wholly or partly on nitrates and should receive at least a portion of the nitrogen fertilizer in this form. The application of all nitrogen fertilizer as nitrate would seldom be advisable because of the instability of this compound in the soil. In many instances ammonium nitrate containing equal amounts of both nitrogen forms may be found a suitable and convenient fertilizer to apply.

It is of great importance in the management of nursery soils to keep in mind the relationship that exists between the total soil fertility, as given in the proposed standards, the fraction of nutrients in the soil solution, and the content of nutrients actually required by seedlings during their 1- or 2-year period of growth.

The amount of a nutrient which is necessary for actual annual metabolism of forest seedlings constitutes, as a rule, but a small fraction of the total available supply of this nutrient present in a productive nursery soil. For example, the amount of calcium annually taken up by the growth of even calciphilous hardwood seedlings is less than 1 milliequivalent per 100 gm., or 400 pounds per acre. Nevertheless, a productive hardwood nursery soil must contain at least 5 milliequivalents per 100 mg. of replaceable calcium, or 2,000 pounds per acre. The presence of this high amount is vital because calcium fulfills numerous functions in soil besides that of a plant nutrient; it promotes aggregation, regulates reaction, counteracts the toxicity of other ions, and stimulates the activity of micro-organisms.

In nursery soils, exposed to rainfall and artificial irrigation, the fraction of nutrients in solution is subject to constant changes. During a period of abundant rainfall, the readily soluble salts are leached and the soil is saturated with nearly pure water. After the rains have stopped, additional nutrients are gradually released into the soil solution by hydrolysis and activity of micro-organisms from the more soluble minerals, exchange material, and organic compounds, i. e., from the storehouses of the plant nutrients (7).

The higher the reserve supply of nutrients, i. e., the level of total soil fertility, the higher, as a rule, is the level of the readily available fraction, the greater is the ability of the soil to resist depletion by leaching or plant feeding, and the greater is the assurance of an uninterrupted and balanced nutrition of seedlings.

Recent experience in several nurseries, located on sandy soils in the Lake States region, affords a striking illustration of the importance of total soil fertility upon the quality of nursery stock and the cost of its production. The summers of 1938 and 1939 were characterized by abundant and frequent rainfalls which caused severe leaching of nutrients, particularly soluble fertilizers applied prior to seeding. Early in July the depletion of plant food was manifested by the yellowing and general stagnation of seedlings as well as transplants. However, on soils of high total fertility the stock regained its color and vigor as soon as the precipitation decreased. On the contrary, on soils of low total fertility the signs of starvation became more and more apparent, and the quality of stock in some cases did not fully recover even at a high cost of liquid fertilizer treatments.

DISCUSSION

As early as the beginning of this century, Hilgard (*5*) repeatedly emphasized the far-reaching importance of analyses of soils supporting productive forest stands. In the preface of his classic text on soils he states:

> The natural floras and sylvas are thus the expression of secular, or rather, millenial experience, which if rightly interpreted must convey to the cultivator of the soil the same information that otherwise he must acquire by long and costly personal experience.

Hilgard (*5, p. 316*) particularly warned against—

> the futile attempts to deduce practically useful results from the chemical analysis of *soils long cultivated*, without first studying the less complex phenomena of *virgin* soils.

The ideas of Hilgard, expressed with reference to agronomical practice, are even more significant in relation to silviculture. The generally accepted concept of Mitscherlich's "optimum" growth arrived at on the basis of dry-matter production (*6*) is not applicable to the production of nursery stock. Stock having the maximum dry weight is too expensive to raise, and often difficult to plant. Such abnormally developed or unreasonably large "forced" seedlings are likely to show decreased vigor and poor survival on depleted and exposed cut-over lands.

The fertility levels, derived from the analyses of natural seedbeds under productive forest stands, should give the closest approach to the physiological optimum of growth conditions; such fertility levels help to prevent not only the starvation of seedlings, but abnormal development and unreasonably high production of dry matter.

The analyses of virgin or nearly virgin soils give not only the amount of nutrients, which may be somewhat variable, but the normal ratio, or proportion of the various constituents, which is of great importance in a balanced nutrition of seedlings.

SUMMARY

The soils under productive stands of representative hardwood species (*Betula lutea, Acer saccharum, Tilia americana, Ulmus americana,* and *Fraxinus americana*) were analyzed for pH value, base-exchange capacity, total and available nitrogen, available phosphorus, available potassium, and replaceable bases. The average values obtained by means of statistical treatment of the results are suggested as standards for the maintenance of fertility in hardwood nursery soils. The analyses of soils showed that the nitrogen-phosphoric acid-potash ratio lies in the proximity of 1–2–5 for yellow birch and 1–3–5 for the rest of the hardwood species studied.

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EFFECT OF ROASTING AND SCALDING PIMIENTO FRUITS IN PREPARATION FOR CANNING ON THE SUBSEQUENT GERMINATION OF THE SEEDS[1]

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INTRODUCTION

Several thousand pounds of seed of the Perfection pimiento (*Capsicum frutescens* L.) are produced annually in Georgia as a byproduct of the canning industry. Some of the seed are sold to seed companies in the United States and some are shipped abroad, but the bulk of the stock is issued each spring by the canners to contract growers, who use the seed for the next year's crop. Since the fruits are either fire-roasted or scalded in oil to remove the skins for canning, the seeds are supposedly exposed to rather high internal temperatures. Most canners core their seed stock by hand before the skins are removed, but the question of the value of the seed from roasted and scalded fruits is being constantly agitated by growers since they can be obtained at little or no expense.

The investigations reported in this paper had as their object (1) to determine the influence of roasting and scalding Perfection pimiento fruits on the incidence and severity of the reduction in seed germination, and (2) to ascertain the types of seedling injury produced. Special attention was given to a study of the internal fruit temperatures to which the seeds are exposed.

Within recent years much has been added to the knowledge of the factors that affect the germination of seeds. So far as the writer has been able to find, however, the literature coutains no work along this ling with peppers.

REMOVAL OF FRUIT SKINS

The two methods used in Georgia for removing the tough outer skins of pimientos prior to canning are not well known outside of the State. Since this investigation deals with the comparative effects of the two methods, a brief description of each is given.

THE ROASTING METHOD

Approximately 70 percent of the pimiento packing plants in Georgia use the roasting method for removing the skins. All the present-day roasters are of the rotary type, consisting of a 20-foot piece of 18-inch

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steel tubing with walls three-eighths of an inch thick and a cork-screw-fashioned baffle running lengthwise on the inside. Each unit is mounted between six rollers on a steel frame having approximately a 3-percent slope and is rotated by an electric motor, which makes it possible to adjust the speed of the roaster as desired. The roasters are fired with either crude oil or natural gas under pressure from a burner mounted at the lower end. This forces the hot flame practically the entire length of the roasters. The temperature of the roasters averages 900° C., and the fruits emerge charred black. After leaving the roasters the fruits are carried down a belt and through a rotary washer under pressure to remove the charred skins and finally into the hands of workmen who core, rewash, and pack them.

THE SCALDING METHOD

The scalding equipment consists of steel vats 2 feet wide and 20 feet long filled with a low-grade cylinder oil having a flasb point of approximately 495° C. and mounted over a large furance. After the oil has attained a temperature of 425° the fruits are elevated into the vat and moved along through the oil by means of several large steel perforated paddles pulled by an endless chain. When the scalded fruits reach the far end of the vat, which takes on an average 42 seconds, they are dumped into a rotary washer where hot water and live steam under pressure remove many of the loosened skins and most of the oil. From the washer the fruits are taken to the packing plant on an endless belt, where they are washed by hand, cored, and packed.

EXPERIMENTAL PROCEDURE

During the 1938 pimiento canning season seed samples from roasted and scalded fruits were collected from 10 packing plants in Georgia for germination tests, and in 1939 similar samples were secured from the same canneries. The check treatment consisted of seeds from freshly picked ripe fruits that had not been heated.

By the use of a Hoskins type PA pyrometer, a minimum of 100 internal temperature readings were made of fruits by quickly inserting the thermocouple wire through the fruit wall into the locules surrounding the seeds at intervals after they had dropped from the roasters and scalding vats.

After the seeds had been removed from the fruits, 10 rows of 100 seeds each of all lots were sown one-half inch deep in flats 3 by 15 by 22 inches filled with a well-composted soil such as is ordinarily used by the growers as a planting medium in the hotbed. The flats were kept reasonably moist, and since the speed of germination of pepper seeds is known to be governed largely by the temperature to which they are subjected (*1*, *2*),[3] that of the greenhouse was maintained as nearly as possible between 70° and 85° F. After the seeds began to germinate, the flats were examined daily to make notes and seedling counts.

EXPERIMENTAL RESULTS

CONDITIONS SURROUNDING SEEDS DURING ROASTING AND SCALDING PROCESSES

Although internal temperature readings of fruits could not be made while the fruits were actually on their way through the roasters and

[3] Italic numbers in parentheses refer to Literature Cited, p. 228.

scalding vats, a knowledge of the temperatures to which they were subjected, the length of time they were exposed, and the subsequent fruit temperature readings (table 1), afforded a good indication of the conditions that surrounded the seeds.

TABLE 1.—*Comparison of average temperatures to which fruits were subjected for removing skins, and their effect upon the average internal temperatures surrounding the seeds*

Method used for removing skins of fruits	Average temperature to which fruits were subjected	Average length of time fruits were exposed	Average internal temperature of fruits— At time of emergence from heating units	Average internal temperature of fruits— 60 feet down belt where cores were removed
	° *C.*	*Seconds*	° *C.*	° *C.*
Fire roasting	900	30	71	45
Oil scalding	425	42	60	47

Table 1 shows that fruits that underwent the roasting process were exposed to an average temperature of 900° C. for 30 seconds. It is not surprising, therefore, that the internal temperature, to which the seeds were exposed, attained a height of 71° C. during the roasting process. Even after the fruits had traveled 60 feet on the belt and had gone through a washer of cold water under pressure, the temperature had dropped only to 45°. While the oil bath of the scalding method was held at approximately 425°, the fruits were 12 seconds longer in going through this medium than through the roasters. When the fruits emerged from the oil they had an average internal temperature reading of 60°, and by the time they reached the coring table the temperature had dropped only to 47°, which was 2° higher than that of the roasted fruits after they had traveled the same distance. This small difference was due largely to the fact that the cooked skins of roasted fruits were removed by running the fruits through a rotating washer of cold water under pressure, thus cooling them suddenly, while those of scalded fruits were removed by the same method but with live steam and boiling water.

SEED GERMINATION

The data in table 2 show that the percentage germination of seeds from both fire-roasted and oil-scalded fruits was relatively low each year as compared with that of seeds from the untreated or check fruits.

TABLE 2.—*Average percentage germination of pimiento seeds from 10 canneries as affected by method of removing skins from the fruits for canning, 1938–39*

Cannery	Seed from fire-roasted fruits 1938	Seed from fire-roasted fruits 1939	Seed from fire-roasted fruits Average	Cannery	Seed from oil-scalded fruits 1938	Seed from oil-scalded fruits 1939	Seed from oil-scalded fruits Average
A	38.1	1.7	19.9	F	30.5	6.6	18.6
B	13.0	24.9	19.0	G	19.5	33.5	26.5
C	4.6	0	2.3	H	16.5	9.6	13.1
D	31.8	7.9	19.9	I	7.3	6.8	7.1
E	2.6	15.7	9.2	J	13.5	.5	7.0
Average [1]	18.0	10.0	14.0		17.5	11.4	14.5
Check	91.3	90.2	90.7				

As a whole the average values for both methods were somewhat lower in 1939 than in 1938. However, upon comparing the 2-year averages it is of special interest to note that they closely agree, that for the roasting method being 14.0 percent and for the scalding method 14.5 percent. The remainder of the seeds were apparently rendered inactive by the heat treatments and failed to emerge. This, as well as figure 1, indicates that one method used for removing the skins of Perfection pimientos for canning was about as harmful to the subsequent germination of the seeds as the other.

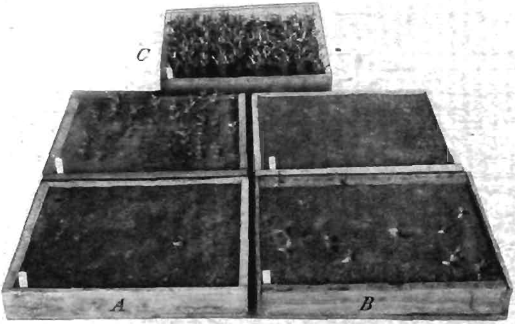

FIGURE 1.—Germination of pimiento seeds from fruits the skins of which were removed by (*A*) fire roasting and (*B*) oil scalding, as compared with checks (*C*).

In studying the data in table 2 in connection with those in table 1, it might seem that pimiento seed should be able to withstand higher temperatures without suffering such drastic reductions in germination. Dry seeds having hard seed coats (the morphology of which has been described by Cochran (*3*)), would, no doubt, react in this manner. In the case of wet seeds, however, attached to the placenta on the inside of the fruits, and consequently in an almost saturated atmosphere, the permeability of the seed coat is thought to be increased by the increase in the surrounding temperature, thus allowing the heat to penetrate and readily reach the embryo. This contention is substantiated by the findings of Denny (*5*) who, in using seeds from six species of plants, found that with some the permeability of the seed coat to water was increased tremendously by heating them.

SEEDLING INJURY

The normal germination process of the Perfection pimiento seed

consists of a small dormant plant surrounded by a thin seed coat. Upon the absorption of water this dormant plant resumes growth and within a relatively short time, under favorable conditions, it develops into a seedling consisting of the primary root, the hypocotyl, two cotyledons, the first pair of true leaves, and the plumule.

From the numerous germination tests that have been conducted on pimiento seeds by the Departments of Botany and Horticulture at the Georgia Agricultural Experiment Station, it has been known for some

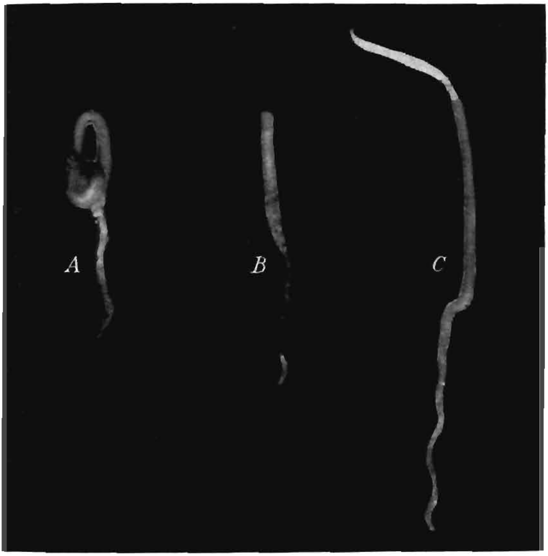

FIGURE 2.—Abnormal seedlings that emerged from pimiento seeds from fruits the skins of which were removed by fire roasting or oil scalding: *A*, Adhering seed coat; *B*, baldhead; *C*, single cotyledon.

time that the heat treatments used for removing the fruit skins result in failure of many of the seeds to germinate. As a whole, however, the growers who have asked about the quality of the seeds from such fruits have not understood that, in addition to low vitability, there may be other types of damage that are not apparent until after germination. This damage usually manifests itself in the form of abnormal seedlings caused by adhering seed coats, baldheads, and single cotyledons (fig. 2). These abnormalities were not classified separately in this study, but collectively 4.7 percent of the seedlings which emerged from the treated

The failure of the seed coat to be released from the cotyledons is thought to be due in some measure, at least, to the heat which causes the embryo to stick to the seed coat; nevertheless, the fact is not overlooked that some of the damage was probably the result of certain defects in the seed coats themselves.

Baldhead is the result of abscission of the cotyledons and plumule, due for the most part to injury caused by the high temperature. Wester and Magruder (*8*) found that disease organisms caused the same condition in seedlings of the Baby Fordhook bush lima bean, while Bainer and Borthwick (*1*) and Harter (*6*) found that in several other types of beans it was due to internal injuries caused by the machinery used for threshing the seeds. On close examination the presence of baldhead in pimiento seedlings could be detected even before the seed coat was shed, as in most cases the severing took place at the base of the cotyledons, leaving only the bare hypocotyl. In beans the injury usually occurred above the cotyledons in the region of the plumule.

Single cotyledon was less prevalent than the two types of injury just described. It was found to be due to a failure of one of the cotyledons to develop further after being heated and not to pathogenic infection.

SUMMARY AND CONCLUSIONS

Fire roasting and oil scalding are the two methods used in Georgia for removing the outer skins of pimiento fruits preparatory to canning. Fruits having passed through the roasters emerged with their seeds exposed to an average internal temperature of 71° C. Fruits just out of the scalding vats had an average internal temperature of 60°, but were 12 seconds longer in passing through the oil medium than were those passing through the roasters.

The average germination of seeds over a 2-year period from roasted fruits was 14.0 percent and from scalded fruits 14.5 percent, indicating that one treatment was about as harmful to germination as the other.

Of the seedlings that emerged from the treated seeds, 4.7 percent showed such abnormalities as adhering seed coats, baldheads, and single cotyledons.

It is concluded from this work that Perfection pimiento seeds taken from either fire-roasted or oil-scalded fruits should neither be offered for sale nor used for planting.

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TWO ADDITIONAL FACTORS FOR RESISTANCE TO MILDEW IN BARLEY[1]

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INTRODUCTION

Since there are physiologic races of barley mildew (*Erysiphe graminis hordei*), the breeder of barley should have available a stock of varieties carrying different genes for resistance to this disease. Then if the distribution of races should shift or if new races of the pathogen should develop, he will have material and information which will enable him to meet the changed requirements for resistance.

In connection with a breeding program to develop mildew-resistant barley (*Hordeum vulgare*) suitable for California, Briggs, in 1932, began a study of the genetics of resistance to this disease. Thus far the genetics of resistance to race 3 of *Erysiphe graminis hordei* in eight resistant barley varieties has been established by Briggs (*1*, *2*),[2] Briggs and Barry (*3*), and Briggs and Stanford (*4*). Hanna, Goldfoil, Algerian, Kwan, and an unnamed variety S. P. I. 45492 were shown to differ from susceptible Atlas in one dominant factor for resistance. Crosses between these five resistant varieties showed that the factors in Hanna, Goldfoil, Algerian, and Kwan were different. S. P. I. 45492, however, had the same factor as Algerian, and the factors in Algerian and Kwan were shown to be linked. These factors have been designated as Ml_h, Ml_g, Ml_a, and Ml_k, respectively. The *Ml* indicates that the factor is for mildew resistance, and the subscript indicates the variety in which the particular factor was first found.

The three varieties Arlington Awnless, Chinerme, and Nigrate were found to have two dominant factors, each differing from the factors mentioned above, and each inherited independently of them. Furthermore, these varieties were found to have at least one factor in common. They were tentatively designated as Ml_x and Ml_y pending further investigation of their identity.

The present investigations deal with the inheritance of resistance to mildew in two additional varieties, Psaknon C. I. 6305 and Duplex C. I. 2433.

MATERIALS AND METHODS

The crosses studied were grown in greenhouse benches at Davis and Berkeley during the winters of 1938–39 and 1939–40. Thirty seeds were planted in rows 30 inches long and 5 inches apart, which usually gave 26 to 30 seedlings. They were inoculated in the three-leaf stage with race 3 of mildew, and classified as to degree of infection according to the system suggested by Mains and Dietz (*6*) and used previously (*1*, *2*, *3*, *4*). No attempt was made to distinguish between types 3 and 4. Plants showing type 4 infection were considered susceptible; those showing types 0, 1, or 2, resistant.

[1] Received for publication May 28, 1940.
[2] Italic numbers in parentheses refer to Literature Cited, p. 236.

Psaknon has given a mildew reading of 1 to 2 in greenhouse trials It gave a reading of 0–1 to Tidd's (*8*) race 6 and a 0 reading to hi race 7. Duplex has given a type 0–1 reaction to race 3 here. It ha given a reading of 1 or less to all the races of Mains and Dietz (*6*) Mains and Martini (*7*), Tidd (*8*), and race A of Honecker (*5*), but a 2–3 reaction to Honecker's race B. Atlas has uniformly given a type 4 reaction to race 3.

Crosses between resistant Psaknon and Duplex and susceptible Atlas were grown to ascertain the number of resistant factors present Crosses with other resistant varieties were grown to determine the identity of such resistant factors. Besides the F_2, the F_3 was grown when necessary. Every tenth row was seeded to Atlas, which always gave a type 4 reaction.

EXPERIMENTAL RESULTS

INHERITANCE OF RESISTANCE TO MILDEW IN HYBRIDS WITH PSAKNON

The F_2 and F_3 generations of the several crosses were grown concurrently. Table 1 shows crosses studied and results obtained.

TABLE 1.—*The classification of parents of F_2 plants and F_3 rows, as indicated, of the crosses named, grown in the greenhouse at Davis, Calif., 1938–39*

Parent or hybrid	Individual plants or rows	Generation	Observed: Resistant	Observed: Heterozygous	Observed: Susceptible	Expected: Resistant	Expected: Heterozygous	Expected: Susceptible	Ratio	Value of *P* for ratios indicated
			Number	*Number*	*Number*	*Number*	*Number*	*Number*		
Atlas	Plants				59					
Psaknon	do		27							
Psaknon × Atlas	do	F_2	349		100	337		112	3:1	>0.1
Atlas	Rows				14					
Psaknon	do		2							
Psaknon × Atlas	do	F_3	31	61	28	30	60	30	1:2:1	>.5
Psaknon	Plants		27							
Hanna	do		27							
Psaknon × Hanna	do	F_2	400		18	391.9		26.1	15:1	>.1
Psaknon	do		29							
Goldfoil	do		28							
Goldfoil × Psaknon	do	F_2	390		25	389.1		25.9	15:1	>.9
Psaknon	do		29							
Arlington	do		28							
Arlington × Psaknon.	do	F_2	418		0					
Psaknon	Rows		2							
Arlington	do		2							
Arlington × Psaknon.	do	F_3	110		0					
Psaknon	Plants		29							
Nigrate	do		26							
Psaknon × Nigrate	do	F_2	458		0					
Psaknon	Rows		2							
Nigrate	do		2							
Nigrate × Psaknon	do	F_3	83		0					
Psaknon	Plants		29							
Algerian	do		30							
Psaknon × Algerian	do	F_2	398		29	400.3		26.7	15:1	>.5
Psaknon	do		58							
Kwan	do		29							
Kwan × Psaknon	do	F_2	743		59	751.9		50.1	15:1	>.1

In the F_2 of Psaknon × Atlas there were 349 resistant and 100 susceptible plants. This result gives a probability greater than 0.1 on the basis of a 3:1 ratio, thus indicating the presence of a single dominant factor for resistance to race 3 of mildew. In F_3 there were 31 resistant, 61 segregating, 28 susceptible rows, all in conformity with expectations.

In the crosses between Psaknon and the four varieties Hanna, Goldfoil, Algerian, and Kwan, each of which is known to have a different factor for resistance, bifactorial ratios were obtained, indicating that the factor found in Psaknon differs from the four factors present in the varieties named above.

No susceptible plants appeared in F_2 or F_3 of Arlington × Psaknon and Nigrate × Psaknon, although Atlas checks invariably gave a type 4 reaction. The factor found in Psaknon must therefore be identical with one of the two factors previously reported in Arlington and Nigrate and temporarily designated as Ml_x Ml_y (*2*). Since the identity of one of these factors has now been established by its presence alone in the variety Psaknon, it will be designated as the Psaknon (Ml_p) factor and hereafter will replace the Ml_x factor designation in Arlington and Nigrate. The Ml_y factors in Nigrate and Arlington may or may not be identical. Until this point is definitely established, the factor will remain under its present designation. Although Chinerme was not crossed with Psaknon, results that will later appear indicate that one of the two factors is the Psaknon factor.

INHERITANCE OF RESISTANCE TO MILDEW IN HYBRIDS WITH DUPLEX

The crosses with Duplex are similar to those described with Psaknon, except that Psaknon was available as a tester for the Ml_p factor. Data from these crosses are recorded in table 2.

TABLE 2.—*The classification of parents or F_2 plants and F_3 rows, as indicated, of the crosses named, grown in the greenhouse at Berkeley, Calif., 1938–39, and Davis, Calif., 1939–40*

Parent or hybrid	Individual plants or rows	Generation	Observed			Expected			Ratio	Value of *P* for ratio indicated
			Resistant	Heterozygous	Susceptible	Resistant	Heterozygous	Susceptible		
			Number	*Number*	*Number*	*Number*	*Number*	*Number*		
Atlas	Rows				107					
Duplex	do		18							
Duplex × Atlas	do	F_3	154	127	8	167.1	117.4	4.5	37:26:1	>0.05
Duplex	Plants		26							
Hanna	do		29							
Duplex × Hanna	do	F_2	360		0					
Duplex	Rows		2							
Hanna	do		2							
Duplex × Hanna	do	F_3	103		0					
Duplex	Plants		29							
Goldfoil	do		55							
Duplex × Goldfoil	do	F_2	1,281		9	1,274.9		15.1	253:3	>.1
Duplex	do		26							
Kwan	do		58							
Duplex × Kwan	do	F_2	1,004		10	1,002.1		11.9	253:3	>.5
Duplex	do		26							
Algerian	do		57							
Duplex × Algerian	do	F_2	960		11	959.6		11.4	253:3	>.9
Duplex	do		58							
Psaknon	do		59							
Duplex × Psaknon	do	F_2	1,208		0					
Duplex	Rows		4							
Psaknon	do		4							
Duplex × Psaknon	do	F_3	105		0					
Duplex	Plants		27							
Chinerme	do		26							
Duplex × Chinerme	do	F_2	683		0					
Duplex	do		27							
Nigrate	do		26							
Duplex × Nigrate	do	F_2	413		0					
Duplex	Rows		2							
Nigrate	do		2							
Duplex × Nigrate	do	F_3	100		0					

Because of lack of seed, the F_2 of Duplex × Atlas was not grown. In F_3, 3 rows of 30 seeds each were planted from each F_2 plant after it had become apparent that comparatively few susceptible progenies existed in this population. It was possible, therefore, to identify more completely progenies segregating for the higher ratios of resistant to susceptible plants. In F_3 there were 154 resistant, 127 segregating, and 8 susceptible progenies, a result that suggests 3 independent factors for resistance, giving a probability of greater than 0.05 on this basis. Because the crosses between Duplex and the other resistant varieties indicate the partial constitution of Duplex, they will be considered next and the Atlas cross later.

In the F_2 and F_3 of Duplex × Hanna and Duplex × Psaknon no susceptible plants occurred, showing that Duplex contains the Hanna and the Psaknon factors. Susceptible plants were found in the F_2 of Duplex × Goldfoil, Duplex × Kwan, and Duplex × Algerian, indicating that the Goldfoil, Kwan, and Algerian factors were not present.

No susceptible plants were found in Duplex × Chinerme and Duplex × Nigrate as was expected, since Duplex and Nigrate contain the Psaknon factor and since, as pointed out, Chinerme probably contains the Psaknon factor.

Returning to the F_3 of Duplex × Atlas: The distribution of these progenies by percentage classes is shown in table 3.

TABLE 3.—*Distribution of parents and F_3 progenies of Duplex × Atlas into 5-percent classes for mildew susceptibility; grown at Berkeley, Calif., 1938–39*

Parent or hybrid	Progenies, with the percentage of susceptible plants indicated											
	0	1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
uplex	18	---	---	---	---	---	---	---	---	---	---	---
tlas	---	---	---	---	---	---	---	---	---	---	---	---
uplex × Atlas	154	33	21	22	13	15	6	8	3	2	---	---

Parent or hybrid	Progenies, with the percentage of susceptible plants indicated—Continued										Total
	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100	100	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
plex	---	---	---	---	---	---	---	---	---	---	18
las	---	---	---	---	---	---	---	---	---	107	107
plex × Atlas	---	---	1	2	1	---	---	---	---	8	289

As pointed out earlier, there are 154 resistant, 127 segregating, and susceptible progenies, a result which agrees satisfactorily with the mbers expected on the basis of 3 independent factors. Two of ese have been identified as the Hanna and Psaknon factors. As own by the occurrence of 4 segregating progenies falling between -80 percent of susceptible plants, the third factor is recessive and refore different from any of those previously discovered. It will designated hereafter as the Duplex (ml_d) factor for mildew stance.

Except the 4 progenies which segregate 1:3, the segregating progenies overlap so that rows resulting from other genotypes cannot be identified. This gives 154 resistant, 123 segregating (61:3, 15:1, 13:3, 3:1), 4 segregating (1:3), and 8 susceptible progenies where 167.9, 107.6, 9.0, and 4.5 are the numbers expected on the basis of three independent factors. The fit between observed and expected would be improved considerably by assuming that the Duplex factor was rather loosely linked with either the Hanna or the Psaknon factor. As will be recalled, however (table 2), the number of susceptible plants obtained in the F_2 of Duplex×Goldfoil, Duplex×Kwan, and Duplex×Algerian all agreed with the numbers expected on the basis of independent factors. A strain of barley is being isolated which will carry the Duplex factor alone and which will be available not only as a tester for the factor but also for establishing its linkage relationship.

SUMMARY AND CONCLUSIONS

The occurrence of the two new genetic factors for resistance to race 3 of barley mildew is reported. The Psaknon (Ml_p) factor found in the Psaknon variety is dominant in effect and is identical with one of the two factors previously reported in Arlington Awnless, Nigrate, and Chinerme. Hereafter Ml_p will replace the Ml_x factor designation tentatively assigned to these three varieties.

A new factor found in Duplex combined with two previously identified factors, Ml_h and Ml_p, is recessive in effect and is designated as the Duplex (ml_d) factor.

The genetics of resistance to race 3 of barley mildew has now been studied at this station in 10 resistant varieties. The factorial composition follows:

Variety	*Factors for resistance to mildew*
Hanna	Ml_h Ml_h.
Goldfoil	Ml_g Ml_g.
Arlington Awnless	Ml_p Ml_p, Ml_y Ml_y.
Chinerme	Do.
Nigrate	Do.
Algerian	Ml_a Ml_a.
S. P. I. 45492	Do.
Kwan	Ml_k Ml_k.
Psaknon	Ml_p Ml_p.
Duplex	Ml_h Ml_h, Ml_p, Ml_p, ml_d, ml_d.

In all, there are seven different factors for mildew resistance—six dominant and one recessive. The number of factors in a single variety varies from one to three. Of the seven identified, two are definitely linked. The other five appear independent, although the Duplex factor may possibly be linked with either the Hanna or the Psaknon.

Additional factors for resistance to mildew may be found as other resistant varieties are investigated or as other races of mildew are used. Tidd (*9*), using race 6, found a single factor for resistance to mildew in a cross between resistant Nepal 595 and susceptible Featherstone. This factor must differ from any of the seven reported above because Nepal is completely susceptible to race 3.

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A SOLUTION OF NORMAL EQUATIONS GIVING THE STANDARD ERRORS OF THE CONSTANTS[1]

By William Dowell Baten

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Several statisticians (*1, 2, 3, 4, 5, 6, 7, 8, 9*)[2] have devised methods for solving normal equations which arise when fitting linear equations to observed data. Fisher (*3, pp. 142–150*) obtained a solution which enables one to find the standard errors of the constants by using his c values. The object of the present paper is to show how to solve for the constants by the use of calculating machines and how to find the c values, used in determining the standard errors of these constants. The material employed for illustrating the method is that used by Fisher to show the dependence of rainfall on longitude, latitude, and altitude.

Assume that the predicting equation for predicting values of y from given values of x_1, x_2, and x_3 is

$$y = b_1x_1 + b_2x_2 + b_3x_3.\quad (1)$$[3]

The normal equations arising from the least squares method are:

$$\Sigma x_1^2 \cdot b_1 + \Sigma x_1x_2 \cdot b_2 + \Sigma x_1x_3 \cdot b_3 = \Sigma x_1y,$$
$$\Sigma x_1x_2 \cdot b_1 + \Sigma x_2^2 \cdot b_2 + \Sigma x_2x_3 \cdot b_3 = \Sigma x_2y,$$
$$\Sigma x_1x_3 \cdot b_1 + \Sigma x_2x_3 \cdot b_2 + \Sigma x_3^2 \cdot b_3 = \Sigma x_3y.$$

By using the values of the summations given by Fisher the above equations become

$$(2)\quad 1{,}934.1b_1 - 772.2b_2 + 924.1b_3 = 1{,}137.4 = \Sigma x_1y,$$
$$(3)\quad -772.2b_1 + 2{,}889.5b_2 - 119.6b_3 = -592.9 = \Sigma x_2y,$$
$$(4)\quad 924.1b_1 + 119.6b_2 + 1{,}750.8b_3 = 891.8 = \Sigma x_3y.$$

Divide each equation by the absolute value of the coefficient of b_1 in it, and we get

$$(5)\quad b_1 - 0.399255b_2 + 0.477793b_3 = 0.588077 = 0.000517036\Sigma x_1y$$
$$(6)\quad -b_1 + 3.741906b_2 + 0.154882b_3 = -0.767806 = 0.00129500\Sigma x_2y$$
$$(7)\quad b_1 + 0.129423b_2 + 1.894600b_3 = 0.965047 = 0.00108213\Sigma x_3y$$

Eliminate b_1 as follows

$$(8) = (5) + (6):\quad 3.342651b_2 + 0.632675b_3 = -0.179729 = 0.000517036\Sigma x_1y + 0.00129500\Sigma x_2y,$$
$$(9) = (6) + (7):\quad 3.871329b_2 + 2.049482b_3 = 0.197241 = 0.00129500\Sigma x_2y + 0.00108213\Sigma x_3y,$$

or

$$(10)\quad b_2 + 0.189273b_3 = -0.0537684 = 0.000154678\Sigma x_1y + 0.000387417\Sigma x_2y.$$
$$(11)\quad b_2 + 0.529400b_3 = 0.0509492 = 0.000334510\Sigma x_2y + 0.000279524\Sigma x_3y.$$

Eliminating b_2 gives

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[1] Received for Publication May 7, 1940. Journal paper No. 452 of the Michigan Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 230.

[3] Where the variables are measured from the mean.

$$0.340127b_3 = 0.104718$$
$$= -0.000154678\Sigma x_1 y - 0.000052907\Sigma x_2 y + 0.000279524\Sigma x_3 y,$$

or

$$b_3 = 0.307879 \quad + \tag{12}$$
$$= -0.000454765\Sigma x_1 y - 0.000155551\Sigma x_2 y + 0.000821822\Sigma x_3 y,$$

or

$$b_3 = c_{13}\Sigma x_1 y + c_{23}\Sigma x_2 y + c_{33}\Sigma x_3 y, \tag{13}$$

where

$$c_{13} = -0.00045477,$$
$$c_{23} = -0.00015555,$$
$$c_{33} = 0.00082182,$$

which are essentially the same as Fisher obtained.

As a check upon the computations, b_3 can be found from (13) by using the values of c_{13}, c_{23}, c_{33} and the values of $\Sigma x_1 y$, $\Sigma x_2 y$, and $\Sigma x_3 y$; this is

$$b_3 = -0.00045477(1{,}137.4) - 0.00015555(-592.9) + 0.00082182(891.8),$$

or

$$b_3 = 0.307876 = 0.30788.$$

Solve for b_2 by substituting the value of b_3 in (11),

$$b_2 = -0.529400(0.30788) + .0509492 = -0.11204$$

$$= 0.529400[-0.000454765\Sigma x_1 y - 0.000155551\Sigma x_2 y + 0.00821822\Sigma x_3 y]$$
$$+ 0.000334510\Sigma x_2 y + 0.000279524\Sigma x_3 y$$

or

$$b_2 = +0.000240753\Sigma x_1 y + 0.000416859\Sigma x_2 y - 0.000155550\Sigma x_3 y,$$

or

$$b_2 = c_{12}\Sigma x_1 y + c_{22}\Sigma x_2 y + c_{23}\Sigma x_3 y, \tag{14}$$

where

$$c_{12} = 0.00024075,$$
$$c_{22} = 0.00041686,$$
$$c_{23} = -0.00015555.$$

The value of b_2 can be checked by the use of (14); this gives

$$b_2 = -0.11204.$$

Equation (7) may be used to find b_1 by substituting the values of b_2 and b_3; this gives

$$b_1 = -0.129423(-0.11204) - 1.894600(0.30788) + 0.96047 = 0.39624$$
$$= -0.129423[0.000240753\Sigma x_1 y + 0.000416859\Sigma x_2 y - 0.000155550\Sigma x_3 y]$$
$$- 1.894600[-0.000454765\Sigma x_1 y - 0.000155551\Sigma x_2 y + 0.000821822\Sigma x_3 y]$$
$$+ 0.00108213\Sigma x_3 y,$$

or

$$b_1 = +0.000830439\Sigma x_1 y + 0.000240756\Sigma x_2 y - 0.000454766\Sigma x_3 y,$$

or

$$b_1 = c_{11}\Sigma x_1 y + c_{12}\Sigma x_2 y + c_{13}\Sigma x_3 y, \tag{15}$$

where

$$c_{11} = 0.00083044,$$
$$c_{12} = 0.00024076,$$
$$c_{13} = -0.00045477.$$

By the use of (15), $b_1 = 0.39624$.

The standard errors of the b's can now be obtained from (13), (14), and (15) by rewriting these equations. For example (13) may be written as follows:

$$b_3=c_{13}[x_{11}y_1+x_{12}y_2+\ .\ .\ .\ +x_{1n}y_n]$$
$$+c_{23}[x_{21}y_1+x_{22}y_2+\ .\ .\ .\ +x_{2n}y_n]$$
$$+c_{33}[x_{31}y_1+x_{32}y_2+\ .\ .\ .\ +x_{3n}y_n],$$

or

$$=(c_{13}x_{11}+c_{23}x_{21}+c_{33}x_{31})y_1+(c_{13}x_{12}+c_{23}x_{22}-c_{33}x_{32})y_2$$
$$+\ .\ .\ .\ +(c_{13}x_{1n}+c_{23}x_{2n}+c_{33}x_{3n})y_n$$
$$=\Sigma(c_{13}x_{1i}+c_{23}x_{2i}+c_{33}x_{3i})y_i.$$

The quantity b_3 is linearly related to the y values, for the x values are fixed. The standard deviation of the values of the y's for any value of x is assumed to be the same for each value of x; that is, the standard deviation of each y array on x is the same. The standard error of b_3 is therefore

$$\sigma b_3=$$
$$S_y\sqrt{c_{13}^2\Sigma x_1^2+c_{33}^2\Sigma x_2^2+c_{33}^2\Sigma x_3^2+2c_{13}c_{23}\Sigma x_1x_2+2c_{13}c_{33}\Sigma x_1x_3+2c_{23}c_{33}\Sigma x_2x_3}$$

where S_y is the standard error of estimate.

By the use of determinants the last expression can be reduced to

$$\sigma b_3=S_y\sqrt{c_{33}}.$$

The standard deviation of b_3 is hence

$$\sigma b_3=4.3327^4\sqrt{0.00082182}=0.12421.$$

In a similar way the standard deviations of b_1 and b_2 can be shown to equal respectively

$$\sigma b_1=S_y\sqrt{c_{11}}=4.3327\sqrt{0.00083044}=0.12486,$$

and

$$\sigma b_1=S_y\sqrt{c_{22}}=4.3327\sqrt{0.00041686}=0.08846.$$

The values of the constants with their standard errors are

$$b_1=0.39624\pm0.12486,$$
$$b_2=-0.11204\pm0.08846,$$
$$b_3=0.30788\pm0.12421.$$

The above method shows how to find the c values and also the regression constants and can be used with modern calculating machines. This method is not only easy to follow, but is also easy to teach to computers.

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THERMAL DECOMPOSITION OF UNDERCURED ALFALFA HAY IN ITS RELATION TO SPONTANEOUS IGNITION[1]

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INTRODUCTION

In a previous investigation (*3*)[2] it was found that the oxidation of natural undercured alfalfa hay in air at temperatures of approximately 77° to 80° C. was accompanied by a consumption of oxygen in excess of the carbon dioxide formed. This fact was attributed to the probable production by heat and chemical action of unsaturated or easily oxidizable substances that were destroyed at once by oxidation without the production of carbon dioxide. On the other hand, the oxidation of undercured alfalfa that had undergone spontaneous heating in the mow resulted in a much smaller loss of oxygen and generally in a production of carbon dioxide greater than the loss of oxygen. This smaller consumption of oxygen was ascribed to the losses of organic matter that occur during spontaneous heating and to the previous oxidation in the mow of any unsaturated substances that might have been formed.

Since these oxidations were conducted under conditions that precluded the possibility of activity by micro-organisms, the conclusion was formed that a consumption of oxygen greater than the production of carbon dioxide in the haymow undergoing spontaneous heating was evidence of chemical oxidation, while the production of carbon dioxide in amounts equal to or greater than the oxygen consumed was ascribed to biological action. The results of the analysis of numerous samples of gas collected from heating haymows interpreted on the basis of this distinction between chemical and biological activity indicated that along with the operation of biological agencies in the heating haymow there occurs also a purely chemical oxidation, shown by a loss of oxygen considerably in excess of the carbon dioxide formed, and that this chemical oxidation is more marked beyond the temperature range usually ascribed to the activity of micro-organisms. This conclusion was found to be in agreement with that of Haldane and Makgill (*2*), who had previously determined the rates of absorption of oxygen and liberation of carbon dioxide in wetted hay at different temperatures by a different procedure.

It was fully recognized that the hypothesis of the production of unsaturated substances in the laboratory oxidation experiments by a purely chemical process and in the heating haymow by chemical processes alone, by the action of micro-organisms alone, or by a combination of both agencies, required verification and that this verification could be accomplished conclusively only by the separation and identification of such substances. Furthermore, if further investiga-

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[2] Italic numbers in parentheses refer to Literature Cited, p. 257.

tion should demonstrate that under the influence of heat supplied from external sources, with the exclusion of microbial activity, unsaturated products are in fact formed, the question would naturally arise whether or not chemical activity in the heating haymow may be due at least in part to the production by heat of unsaturated substances readily susceptible to oxidation, that is, whether the formation of these substances may be the result of the heat of microbial action rather than the direct result of the activity of micro-organisms.

The investigation reported in this paper was undertaken to obtain further evidence of the formation of unsaturated substances, without the intervention of micro-organisms, in undercured alfalfa hay subjected to heat in an inert atmosphere.

PLAN OF THE INVESTIGATION

Briefly stated, the investigation consisted in: (1) Determining the oxygen absorbed and the carbon dioxide formed in the heating of undercured alfalfa (*Medicago sativa*) hay in a definite volume of air at a temperature slightly above that at which micro-organisms exist; and (2) determining the same factors, under conditions as nearly as possible identical, for duplicate samples of the alfalfa after it had been heated in an atmosphere of nitrogen for an arbitrary length of time. The relative oxygen absorption and carbon dioxide formation thus determined were expected to show the effect on alfalfa of heating in an inert atmosphere.

ALFALFA USED

Alfalfa A.—This was first-cutting alfalfa collected from the field nearly 3 years before and stored in large airtight fruit jars in a refrigeration room at 30° F. When used, it contained 36.35 percent of moisture. Samples of 40 gm. each were used, equivalent to 25.46 gm. of dry matter. The dry matter contained 7.42 percent of ash.

Alfalfa B.—This was first-cutting alfalfa collected from the field 7 months before and kept in large airtight fruit jars at 30° F. It contained 39.6 percent of moisture. The 40-gm. sample used was equivalent to 24.16 gm. of dry matter. The dry matter contained 8.08 percent of ash.

Alfalfa C.—This was first-cutting alfalfa collected from the field) months before and kept in large airtight fruit jars at 30° F. until ısed. It contained 36.65 percent of moisture; 8.17 percent of ash, ›n the dry basis; and 25.34 gm. of dry matter per 40-gm. sample.

Alfalfa D.—This was third-cutting alfalfa partly dried in the sun or 6 hours and then air-dried indoors for 16 hours to a moisture ontent of 42.41 percent. It had been stored in airtight jars in a efrigeration room at 20° F. for about 3 weeks before it was used. A 0-gm. sample contained 23.04 gm. of dry matter. The dry matter ad an ash content of 7.52 percent.

Alfalfa E.—This was first-cutting alfalfa stored in a refrigeration ›om at 30° F. for approximately 2 years and 3 months before use. ; contained 33.82 percent of moisture. A 40-gm. sample contained ;.47 gm. of dry matter, and the dry matter contained 8.13 percent ash.

Alfalfa F.—This was third-cutting alfalfa from the same lot as falfa D, but it was nearly 6 months older at the time of the experi- ent. It contained 40.10 [illegible]

contained 23.96 gm. of dry matter, and the dry matter contained 7.52 percent of ash.

APPARATUS AND METHODS

The apparatus in which these experiments were conducted was essentially the same as that used in the preceding laboratory oxidation experiments (*3*) with the exception of the improved constant-temperature bath in which the oxidation flask and contents were heated. This bath, 40 cm. in diameter and 30 cm. deep, was provided with electric immersion heaters, a three-heat switch, and a mercury thermoregulator. In this bath the temperature of the heavy mineral oil surrounding the oxidation flask could be maintained constant to a few tenths of a degree. Inasmuch as the temperature of the samples of alfalfa and the air in the flask was always somewhat lower than the temperature of the bath, owing to the moisture in the samples, to attain the temperatures in the flask as recorded for the experiments it was necessary to maintain the temperatures of the bath approximately 5° higher.

The oxidation flasks varied slightly in capacity, but the same flask was used throughout a given experiment, except in one case, and in this the effect was apparently negligible. The enclosed volume of air or nitrogen, later referred to as the volume of the assembly, was that gas enclosed in the flask and connections between the stopcocks above the two mercury containers (*3, fig. 3*) at the time of closing the assembly, corrected for the volume occupied by the hay sample. This volume was corrected for aqueous tension, and this corrected volume was computed to the arbitrary standard conditions of 25° C. and 760-mm. pressure. Consequently, the original volume of gas given in table 1 varies somewhat in each of the several experiments. The capacity of the flasks ranged from 1,037 to 1,055 cc., while the volume of the assembly ranged from approximately 1,045 to 1,067 cc. less the volume of the sample.

A 40-gm. sample was placed loosely in the oxidation flask, and the flask was tightly closed by means of the rubber stopper carrying the thermometer, the condenser, and the capillary tube. The projecting end of the capillary tube and the upper end of the condenser tube were securely connected to their respective places in the assembly by means of tightly fitting rubber tubing of the best quality. In an oxidation operation, the heating was begun at once. When the temperature of the bath had risen to within a few degrees of the temperature at which it was to be maintained, the heating was continued only with the heating coil controlled by the thermoregulator. As the temperature rose, the air displaced by expansion was collected in the mercury container connected to the condenser tube, and throughout the period of heating the gas was maintained at atmospheric pressure. In approximately 1 hour the temperature selected for the initial temperature of the arbitrary period of heating, 4 hours in the comparative tests, was reached. The temperature rose in time to the maximum for the experiment, and more frequently than otherwise dropped slightly before the heating operation was completed. At the end of the 4-hour peroid of heating, the heater was cut off, and the bath was cooled as rapidly as possible to a temperature suitable for mixing and sampling the gas by the rapid passage of cold water through the

cooling coil provided for this purpose. Throughout the entire experiment the condenser tube connected with the flask was kept cold in order to retain as constant as possible the moisture content of hay and flask.

After the enclosed gas had been thoroughly mixed by passing it back and forth from one container to the other through the flask, sufficient gas was drawn into one of the containers for several analyses. Usually this reduced the pressure in the apparatus, but it did not cause any leaking, as proved by the constancy of the high degree of vacuum indicated on the manometer after the residual gas had finally cooled to room temperature. From the analysis of the gas after oxidation, the volume of this gas was computed on the basis of the volume of nitrogen in the original volume of air in the assembly, as were also the volumes of carbon dioxide and oxygen. This computation provided the data necessary for the determination of total change in volume, carbon dioxide formed, and oxygen consumed.

The carbon dioxide formed and the oxygen lost by the alfalfa under these conditions having thus been determined, a duplicate sample of the alfalfa was taken for preheating in the nitrogen. This nitrogen contained about 0.4 percent of oxygen, but inasmuch as the alfalfa samples all appeared to contain some enclosed air, and some of them perhaps also carbon dioxide, removal of the small proportion of oxygen from the nitrogen was not considered essential, as it would not have produced an atmosphere entirely free from oxygen. Occlusion of air appears also to be responsible for the apparent increase in the oxygen content of the nitrogen in some of the experiments after preheating the alfalfa in it. The actual amounts of oxygen in the assembly as found by analysis are given in table 1.

The air in the assembly containing the sample was displaced as completely as practicable by passage of a slow stream of nitrogen through the assembly for 10 to 12 hours, during which time cold water was passed rapidly through the condenser to prevent loss of moisture from the hay. Then an extra supply of nitrogen was collected in one of the mercury containers, which, when well mixed with the nitrogen in the assembly, was sufficient to provide an excess of the mixture for analysis. The assembly was then closed at atmospheric pressure, and the temperature of the enclosed gas was recorded.

The operation of heating the hay in the nitrogen was then begun and continued for the desired length of time according to the procedure previously outlined for the oxidation operation. At the conclusion of this operation a portion of the gas mixture was drawn off for later analysis. Then the partial vacuum produced was relieved by the introduction of fresh nitrogen, and again nitrogen was passed through the assembly, for 2 to 3 hours, to reduce the carbon dioxide content and to increase the proportion of nitrogen. After this procedure an excess of nitrogen was introduced to provide as before for analysis of the mixture. From the results of this analysis was computed the volume of oxygen to be added to the volume of gas in the assembly, closed off under prevailing conditions of temperature and pressure, to bring the oxygen content of the resultant mixture to exactly 20.86 percent. This volume of oxygen, measured under conditions of temperature and pressure identical with those of the gas in the assembly, was then added to and mixed well with the latter gas. The

assembly was again closed, and oxidation with the known volume of this synthetic air for the 4-hour period was conducted as previously described. Analysis and computations were also made as before.

In experiments 1 to 5, inclusive, the three successvie operations outlined above were conducted in the following order:

(*a*) Heating 40 gm. of the alfalfa for 4 hours in air.

(*b*) Heating 40 gm. of the same alfalfa in nitrogen for 20 hours except in experiment 5.

(*c*) Heating the product of operation (*b*) for 4 hours in air.

Experiments 6 to 9, carried out in a similar manner, were designed to provide additional information regarding the effect of the several heating operations.

Experiment 10 was designed to show the effect of the presence of small quantities of ammonia on the oxidation of hay.

RESULTS

The results of the investigation are shown in table 1. In the second column of the table the temperature range for each operation is given. It will be noted that in experiments 1 to 5 the difference in maximum temperatures for operations (*a*) and (*c*) in four of the experiments ranged from 0.1° to 1.2°, while in one the difference was 2.7°. Inasmuch as the bath temperature varied only slightly, the differences must be attributed to changes in the alfalfa resulting from heating it in nitrogen. The effect of this variation in maximum temperature is probably negligible in experiments 1, 3, and 4; it may have been of slight consequence in experiment 5; and most probably it influenced the results in experiment 2. In both experiments 2 and 5 the effect of the lower temperatnre in operation (*c*) undoubtedly was unfavorable to an increased oxygen consumption over the oxygen consumption in operation (*a*).

Table 1.—*Results of laboratory experiments on the oxidation of undercured alfalfa hay before heating and after heating in an atmosphere of nitrogen*

Experiment No. and alfalfa used	Heat treatment	Original volume of gas (25° C., 760 mm.) (A)	Composition of original gas: CO_2 (B)	Composition of original gas: O_2 (C)	Composition of original gas: N_2	Volume of gas after heating based on N_2 of original gas (D)	Composition of gas after heating: CO_2 (E)	Composition of gas after heating: O_2 (F)	Composition of gas after heating: N_2	Loss or gain in gas volume[1] (A)-(D)	Available O_2 at maximum temperature	CO_2 formed (E)-(B)	O_2 absorbed[1] (C)-(F)	CO_2 formed per 25 gm. of dry matter	O_2 absorbed per 25 gm. of dry matter[1]	Ratio of CO_2 formed to O_2 absorbed
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	
1. Alfalfa A:																
(a) 25.46 gm. of dry matter.	4 hours in air at 71.6°–77.0° C.	1,015.90	.00	211.92	803.98	1,031.67	45.39	182.30	803.98	+15.77	108.41	45.39	29.62	44.57	29.08	1.532
(b) Do.	20 hours in nitrogen at 75.0°–76.5° C.	1,005.80	4.22	18.91	982.67	1,043.95	46.25	15.03	982.67	+38.15	...	42.03	3.88	41.27	3.81	...
(c) Product of heating (b) in nitrogen.	4 hours in air at 75.3°–76.3° C.	1,011.32	28.52	210.86	771.94	988.46	66.13	150.39	771.94	22.86	110.85	37.61	60.47	36.93	59.38	.622
2. Alfalfa B:																
(a) 24.16 gm. of dry matter.	4 hours in air at 76.8°–79.1° C.	1,025.99	.00	214.02	811.97	1,021.22	53.41	155.84	811.97	4.77	102.10	53.41	58.18	55.27	60.20	.918
(b) Do.	20 hours in nitrogen at 76.0°–77.4° C.	989.62	1.98	1.19	986.45	1,036.51	47.06	3.00	986.45	+46.89	...	45.08	+1.81	46.65	+1.87	...
(c) Product of heating (b) in nitrogen.	4 hours in air at 75.0°–76.4° C.	967.93	25.07	202.20	740.66	940.41	55.59	144.16	740.66	27.52	105.20	30.52	58.04	31.58	60.06	.526
3. Alfalfa C:																
(a) 25.34 gm. of dry matter.	4 hours in air at 76.0°–78.3° C.	1,021.93	.00	213.17	808.76	1,017.95	46.83	162.36	808.76	3.98	103.57	46.83	50.81	46.20	50.13	.921
(b) Do.	20 hours in nitrogen at 76.0°–77.9° C.	986.94	1.58	10.95	974.41	1,043.04	56.22	12.41	974.41	+56.10	...	54.64	+1.46	53.91	+1.44	...
(c) Product of heating (b) in nitrogen.	4 hours in air at 76.0°–78.2° C.	994.60	29.64	207.47	757.49	966.50	66.06	142.95	757.49	28.10	101.95	36.42	64.52	35.93	63.65	.564
4. Alfalfa D:																
(a) 23.04 gm. of dry matter.	4 hours in air at 76.0°–77.0° C.	985.65	.00	205.61	780.04	978.23	46.17	152.02	780.01	7.42	105.90	46.17	53.59	50.10	58.15	.862
(b) Do.	20 hours in nitrogen at 76.6°–77.6° C.	983.85	.79	1.57	981.49	1,029.25	43.64	4.12	981.49	+45.40	...	42.85	+2.55	46.50	+2.77	...
(c) Product of heating (b) in nitrogen.	4 hours in air at 76.0°–78.0° C.	971.36	6.80	202.92	761.64	940.41	34.23	144.54	761.64	30.95	100.73	27.43	58.38	29.76	63.35	.470
5. Alfalfa E:																
(a) 26.47 gm. of dry matter.	4 hours in air at 77.0°–78.0° C.	953.30	.00	198.86	754.44	958.14	59.12	144.58	754.44	+4.84	102.49	59.12	54.28	55.84	51.27	1.089
(b) Do.	91¼ hours in nitrogen at 75.0°–82.0° C.	977.83	.79	2.84	974.21	1,105.55	129.57	1.77	974.21	+127.72	...	128.79	1.07	121.64	1.01	...
(c) Product of heating (b) in nitrogen.	4 hours in air at 76.2°–76.8° C.	973.92	.00	203.16	770.76	951.44	35.11	145.57	770.76	22.48	103.72	35.11	57.59	33.16	54.39	.610

. Alfalfa D:																
(*b*) 23.04 gm. of dry matter.	4 hours in nitrogen at 76.0°–77.8° C.	993.85	1.29	2.98	989.58	1,017.35	22.69	5.08	989.58	+23.50	------	21.40	+2.10	23.22	+2.28	------
(*c*) Product of heating (*b*) in nitrogen.	4 hours in air at 76.0°–77.0° C.	981.98	1.28	204.84	775.86	955.49	28.28	151.35	775.86	23.49	104.19	27.00	53.49	29.30	58.04	.505
. Alfalfa D:																
(*b*) 23.04 gm. of dry matter.	4 hours in nitrogen at 76.0°–78.2° C.	1,004.43	3.11	6.03	995.29	1,026.18	23.19	7.70	995.29	+21.75	------	20.08	+1.67	21.79	+1.81	------
(*c*) Product of heating (*b*) in nitrogen.	4 hours in nitrogen at 76.0°–77.6° C.	980.90	3.92	7.95	969.03	990.63	14.17	7.43	969.03	+9.73	------	10.25	.52	11.12	.56	------
. Alfalfa D: 23.04 gm. of dry matter.	20 hours in air at 76.0°–78.8° C.	964.08	.06	201.11	762.97	960.92	99.91	98.01	762.97	3.16	98.91	99.94	103.10	108.44	111.87	.069
. Alfalfa B: 24.16 gm. of dry matter.	50 hours in nitrogen at 76.0°–77.5° C.	978.82	1.96	.98	975.88	1,044.50	65.80	2.82	975.88	+65.68	------	63.84	+1.84	66.06	+1.90	------
0. Alfalfa F:																
(*a*) 23.96 gm. of dry matter.	4 hours in air at 75.5°–78.8° C.	1,016.41	.00	212.02	804.39	1,019.09	43.68	171.02	804.39	+2.68	101.38	43.68	41.00	45.58	42.78	1.065
(*b*) Duplicate of (*a*), treated with ammonia.	4 hours in air at 76.0°–79.4° C.	1,023.56	.00	213.51	810.05	988.56	29.22	149.29	810.05	35.00	99.34	29.22	64.22	30.49	67.01	.455

[1] Increase of gas volume is indicated by plus (+) sign.

The results of the first five experiments all show that the ratio of carbon dioxide formed to oxygen absorbed was less in the samples previously subjected to heating in nitrogen, that is, it was less in (*c*) than in (*a*). The fact that the ratio of carbon dioxide formed to oxygen absorbed is greater than unity in experiments 1 (*a*) and 5 (*a*) no doubt is due to some decomposition, perhaps oxidation, during the 3 and 2 years, respectively, in which the hay was kept in cold storage. Furthermore, with the possible exception of experiment 2, in which the conditions were unfavorable to increased oxidation, the results show definitely that the heating of the undercured alfalfa in an atmosphere of nitrogen caused an increase in its oxygen-absorption property.

The oxidations in the first five experiments were conducted over a 4-hour period. Experiment 8 shows a greatly increased oxidation and carbon dioxide production over a period of 20 hours. Experiment 9 when compared with experiment 2 (involving the same alfalfa) shows the effect of heating in nitrogen for 50 hours instead of 20.

In order to obtain information required for a better understanding of the results of experiment 4, and perhaps also of the results of the other experiments, experiments 6 and 7 were carried out as indicated. In experiment 6 the alfalfa was heated for only 4 hours in nitrogen and then for 4 hours in air. The result was an oxygen consumption less than that in experiment 4 (*c*) but practically the same as that in experiment 4 (*a*). Moreover, the total carbon dioxide formed in experiment 6 (*b*) and (*c*) was not much greater than that in experiment 4 (*a*). It thus appears that the 4-hour heating in air in experiment 4 (*a*) produced an effect on the alfalfa practically equivalent to the combined effect of the 4-hour heating in nitrogen followed by the 4-hour heating in air, and in both cases the oxygen consumed was in excess of the carbon dioxide formed. These facts indicate that in the 4-hour heating in air unsaturated substances were produced in amounts practically identical with those produced by the 4-hour heating in nitrogen followed by the 4-hour heating in air. In the former case these substances were oxidized almost simultaneously in the same operation in which they were produced.

Since in experiment 6 (*b*), 23.22 cc. of carbon dioxide was formed in the 4-hour heating in nitrogen, approximately this volume of carbon dioxide may reasonably be assumed to be the result of heat decomposition in experiment 4 (*a*), leaving a difference (50.10–23.22) of 26.88 cc. of carbon dioxide to be accounted for at the expense of the oxygen consumed.

In experiment 7, a duplicate sample of the alfalfa was heated for 4 hours in nitrogen and then for an additional 4 hours in fresh nitrogen. In carbon dioxide production, operation (*b*) agreed closely with operation (*b*) in experiment 6, while operation (*c*) yielded less than one-half as much carbon dioxide (11.12 cc.) The latter volume may reasonably be supposed to approximate the volume of carbon dioxide formed in experiment 4 (*c*) as the result of heat alone, independent of oxidation. But it must be emphasized that this is only an assumption, because in experiment 4 operation (*c*) had been preceded by a 20-hour heating in nitrogen, while in experiment 7 operation (*c*) had been preceded by only a 4-hour heating in nitrogen. However, on this assumption the difference between the carbon dioxide formed in experiment 4 (*c*) and

that in experiment 7 (*c*) (29.76–11.12) may be considered as carbon dioxide formed by an equal volume of oxygen. From the data thus derived a method is suggested for estimating the approximate volume of oxygen absorbed independent of carbon dioxide formed by oxidation in the respective operations of experiments 4 and 6. On the same assumption, similar relations may be pointed out in experiments 1, 2, 3, and 5, although in these experiments the assumption is less warranted because alfalfa different from alfalfa D was used. Table 2 contains an abbreviated presentation of the pertinent contents of table 1 interpreted on the basis of this assumption.

TABLE 2.—*Interpretation of pertinent data from table 1*

Experiment No. and alfalfa used	Heat treatment	O_2 absorbed by 25 gm. of dry matter[1] (A)	CO_2 formed by 25 gm. of dry matter (B)	Ratio of 2 to 1	O_2 absorbed in excess of CO_2 formed[2] (A)−(B)	CO_2 formed by heat alone (C)	CO_2 *formed* at the expense of an equal volume of O_2 (B)−(C)=(D)	O_2 absorbed without production of CO_2 (A)−(D)
		Cc.	*Cc.*		*Cc.*	*Cc.*	*Cc.*	*Cc*
1. Alfalfa A:								
(a)	4 hours in air	29.08	44.57	1.532	−15.49	[3] 22.50	22.07	7.01
(b)	20 hours in nitrogen	3.81	41.27					
(c)	4 hours in air	59.38	36.93	.622	22.45	[4] 11.12	25.81	33.57
2. Alfalfa B:								
(a)	4 hours in air	60.20	55.27	.918	4.93	[3] 22.50	32.77	27.43
(b)	20 hours in nitrogen	+1.87	46.65					
(c)	4 hours in air	60.06	31.58	.526	28.48	[4] 11.12	20.46	39.60
3. Alfalfa C:								
(a)	4 hours in air	50.13	46.20	.921	3.93	[3] 22.50	23.70	26.43
(b)	20 hours in nitrogen	+1.44	53.91					
(c)	4 hours in air	63.65	35.93	.564	27.72	[4] 11.12	24.81	38.84
4. Alfalfa D:								
(a)	4 hours in air	58.15	50.10	.862	8.05	[3] 22.50	27.60	30.55
(b)	20 hours in nitrogen	+2.77	46.50					
(c)	*4 hours in air*	*63.35*	*29.76*	*.470*	*33.59*	*[4] 11.12*	*18.64*	44.71
5. Alfalfa E:								
(a)	4 hours in air	51.27	55.84	1.089	−4.57	[3] 22.50	33.34	17.93
(b)	91¼ hours in nitrogen	1.01	121.64					
(c)	*4 hours in air*	*54.39*	*33.16*	*.610*	21.23	*[4] 11.12*	22.04	32.35
6. Alfalfa D:								
(a)—experiment 4	4 hours in air	58.15	50.10	.862	8.05	[3] 22.50	27.60	30.55
(b)	*4 hours in nitrogen*	+2.28	23.22					
(c)	4 hours in air	58.04	29.30	.505	28.74	[4] 11.12	18.18	29.86
7. Alfalfa D:								
(b)	4 hours in nitrogen	+1.81	21.79					
(c)	4 hours in nitrogen	.56	11.12					

[1] Plus sign indicates gain in volume.
[2] Minus sign indicates CO_2 formed in excess of O_2 absorbed.
[3] Mean of 6 (b) and 7 (b).
[4] From 7 (c).

In column 6 of table 2 are given the volumes of oxygen absorbed in excess of carbon dioxide formed. On the assumption that all carbon dioxide is produced at the expense of an equal volume of oxygen, these volumes become the volumes of oxygen absorbed without production of carbon dioxide, that is, the measure of the oxygen-absorption property of the alfalfa.

The values given in column 7 for experiments 4 and 6 were determined experimentally. The value 22.50 is the mean of experiment 6 (*b*) and experiment 7 (*b*), column 4, and 11.12 is the value determined for experiment 7 (*c*), column 4. For the other experiments these

values are only assumed as approximate, since they were not determined experimentally for the particular alfalfa involved.

The volumes given in column 8 are volumes of carbon dioxide formed at the expense of an equal volume of oxygen, based on the volumes of column 7. The volumes in column 9 then become the volumes of oxygen absorbed without production of carbon dioxide, that is, the measure of the oxygen-absorption property.

The values for oxygen absorbed in excess of carbon dioxide formed (column 6) and those for oxygen absorbed without production of carbon dioxide (column 9) show consistently that a decided increase in its oxygen-absorption property results from the heating of the alfalfa in nitrogen.

The differences in oxygen absorption of the hay before and after such treatment, shown in column 9, are less than those in column 6, but nevertheless the former are quite significant.

The effect of the presence of small quantities of ammonia on the absorption of oxygen is shown in experiment 10 (table 1). A 40-gm. sample of alfalfa F was heated in air for 4 hours according to the usual procedure (experiment 10 (*a*)). A duplicate sample then was taken and placed in a flask (experiment 10 (*b*)). A glass bulb of 2-cc. capacity, with a long capillary outlet, containing approximately 0.8 gm. of ammonia (specific gravity, 0.90), was carefully inserted into the loose mass of alfalfa, with the capillary outlet projecting above the surface of the hay. No escape of ammonia from the bulb could be detected before the assembly was closed and heating was begun. At the conclusion of the experiment the volume of liquid in the bulb did not appear to be greatly decreased, and it still retained a strong ammonia odor. The gas collected for analysis contained 1.40 percent of ammonia gas, which was removed from the samples analyzed before the determinations of carbon dioxide and oxygen were made. Table 1 shows the greatly increased absorption of oxygen under the influence of the ammonia.

SIGNIFICANCE OF RESULTS IN RELATION TO SPONTANEOUS IGNITION

Despite some divergence of opinion regarding the relative influence of the several factors involved, it is generally agreed among students of the subject that the initial and early stages of the spontaneous heating of undercured or wet hay are due mainly to the respiration process of the living plant cells and the activity of micro-organisms, and it has been proved that along with these biological activities there occurs a purely chemical oxidation, which is a contributing factor in the production of heat. These biological activities are unquestionably capable of raising the temperature of the hay to approximately 70° C., or slightly higher, perhaps in some instances to 80°. But at such temperatures the activity of micro-organisms necessarily must cease because of the death of these organisms. Consequently, it is necessary to seek some other explanation to account for the rapid rise of temperature above the limits of micro-organic activity, which occurs when haymows are ignited.

The earliest chemical theory advanced to account for the spontaneous combustion of hay is based on two well-known principles. The first is the fact established by Döbereiner that hydrogen gas

ignites when brought into contact with finely divided platinum. The simultaneous condensation of air and inflammable gases within the pores and on the large surface of platinum sponge, or platinum black, results in such intimate contact that ignition occurs. The second principle is that of pyrophoresis, the behavior of a substance in the so-called pyrophoric condition, that is, very minute subdivision, by virtue of which it is capable of extremely rapid oxidation.

The application of these principles to the explanation of the spontaneous combustion of hay was first suggested by Büchner (*Ranke, 7, p. 361*) in 1872. His explanation is now known as the pyrophoric carbon theory. Because this theory in its essential principles provides perhaps the most reasonable basis for an approach to the solution of the problem, in this paper the discussion of theories of spontaneous combustion are confined to a brief outline of the development of the pyrophoric carbon theory and to an attempt to point out and correlate later views and observations that have led to other explanations involving principles similar or closely related to those underlying this theory.

The most prominent exponent of the pyrophoric carbon theory was Ranke (*7*), who subjected the theory to critical laboratory tests. Ranke also had the good fortune to observe an actual case of the spontaneous combustion of hay, and because of his scientific training was able to describe this occurrence in practically all its essential details in such a manner that his description remains today as perhaps the best account of this phenomenon. He concluded that the spontaneous ignition of the hay was due to the strong oxygen-absorption power of the carbonized hay.

By carbonizing hay at 250° to 300° C., in the laboratory, Ranke was able to obtain a product that on exposure to air would heat to redness. He found, however, that if the hay was heated sufficiently to remove all volatile matter, the charred mass would show no pyrophoric property. From this observation he concluded that very probably these products of destructive distillation played a part in causing spontaneous ignition analogous to that of unsaturated oils in the self-ignition of oily waste. To account for the high temperature of 250° to 300° in the haymow, which was supposed to be necessary for the formation of pyrophoric carbon, he pointed out the effective insulating properties of the densely packed hay, which prevented any great loss of heat by radiation.

According to Ranke, the condition of the burnt mass constituting the core of the mow of burning hay observed by him was that of actual carbon. Apparently this observation and the fact that he had produced a similar carbonized mass in his laboratory experiments led to the supposition that a temperature of at least 250° C. was required to form pyrophoric carbon in the mow. This conception of the necessity of a very high temperature for the formation of pyrophoric carbon undoubtedly greatly retarded the subsequent development of the pyrophoric carbon theory. But the difficulty of explaining such high temperatures led other investigators to the view that carbonization can be produced at much lower temperatures.

Miehe (*5*), a supporter of the low-temperature carbonization theory, expressed his belief that carbonization of hay may take place at 70° C. over a long period with the same result that is attained at higher

temperatures in a short period. He suggested that this carbonization might occur by a process of dry distillation. Also he stressed the necessity of determining whether micro-organisms provide only the heat or also the easily oxidizable compounds that participate in the conditions causing spontaneous ignition. He opposed the view that ordinary heating in haymows is due to simple chemical activity, but he suggested that when the temperature rose to above approximately 70° some physical or chemical change had taken place in consequence of the long-continued effect of heat or bacterial action at 70° which rendered the hay susceptible to purely chemical oxidation.

A similar suggestion was made later by Truninger (*9*), who pointed out the desirability of investigating whether the activity of certain bacteria and molds in the fermenting haystack may produce easily oxidizable substances that might serve as the source of subsequent purely chemical processes.

A clue to the explanation of the rapid rise in temperature above the limits of bacterial activity in the haymow, which ultimately leads to ignition, is given in the theory developed by Browne (*1, pp. 25, 26*) for the spontaneous heating and ignition of large masses of hay. This theory, which was suggested by the simpler and purely chemical example of the spontaneous ignition of oil-soaked cotton waste,

> is based upon the preliminary production by micro-organisms under more or less perfect anaerobic conditions of unsaturated, highly unstable, intermediate-fermentation products upon the surfaces of the porus, cellular materials (the condition being therefore similar to that of the oil-coated cotton). The duration of existence of these readily oxidizable fermentation products is dependent upon the quantity of air that can gain access to the fermenting mass of hay and also upon the quantity of moisture which is present to serve as a reacting medium. If the heaps are small or of open, loose structure the intermediary compounds are destroyed almost as soon as formed, with the result that when vegetative micro-organic life is all destroyed at 70° to 80° C., there is not a sufficient residue of such easily oxidizable, unsaturated substances to carry the production of heat to higher limits. The heat of the microbial life period is probably owing in large part to the oxidation of the same intermediary unstable products that participate in the elevation of temperature above 80° * * *. In other words, the micro-organisms simply produce the highly unstable compounds whose subsequent oxidation, like that of the unsaturated oil upon cotton, generates the increasing quantities of heat that lead first to the destruction of the organisms themselves and then eventually to the ignition of the hay.

Considerable confusion appears in discussions of pyrophoric carbon because of failure to distinguish clearly between so-called pyrophoric carbon, or hay carbon, and hay that has undergone a more limited decomposition with every appearance of carbonization. The first, which is the product of severe carbonization of hay, may approximate true carbon and is in a condition to ignite almost immediately on exposure to the air. The second does not ignite at once on exposure to the air, but is capable of a progressively rapid oxidation that can result in ignition. The description of the carbonized hay of Ranke as pyrophoric carbon also was rather unfortunate, and Ranke himself must have been fully aware that the product of his laboratory experiments, which still retained volatile empyreumatic substances upon which its pyrophoric properties seemed in part to depend, could not have been carbon. The highly carbonized matter constituting the core of the burning mow observed by Ranke and described by him as actual carbon no doubt had undergone a temperature close to ignition

temperature, but it should have been obvious that some of the decomposed hay in other sections of the mow had acquired a pyrophoric condition perhaps long before that temperature had been reached. In fact, he observed that a load of deep-brown rowen drenched with water during its removal from the mow ignited repeatedly when spread out on the grass near a pond and finally was completely consumed. Very probably by the terms "actual carbon" and "pyrophoric carbon" Ranke meant a very highly but not completely carbonized product of hay as a whole or a true pyrophoric carbon intermingled with the product of a lower degree of carbonization.

In the opinion of Truninger, the laboratory determination of the minimum temperature required to produce hay carbon cannot be relied upon conclusively because such experiments can realize only imperfectly the natural conditions in the heating mow. By warming finely ground hay in a dish in a drying oven up to 163° C., he produced a light-brown material, which began to glow at one narrowly defined zone on its surface. When the thin glowing layer was removed and the remaining material was mixed, the latter began to carbonize and after a few minutes was in full glow. In Truninger's opinion, however, it is not impossible that after sufficiently long heating at 90° to 95°, in the presence of strongly acid vapors, self-inflammable carbon may be formed in the mow at a temperature only a little higher, perhaps 120°. He refers to an instance reported to him in which hay of normal appearance pitched to the ground during the demolition of a spontaneously ignited haystack suddenly caught fire with almost explosive violence. He believed that this hay was normal, properly fermented hay which had been brought to a pyrophoric condition by the dry heat of a neighboring fire pocket but that hay which has been rendered pyrophoric only by excessive fermentation and has itself produced the heat necessary for this condition will always exist in a state of carbon formation. Hay that has been heated to 110° is already on the point of ignition, although the actual ignition point determined experimentally lies between 220° and 225°. The temperature interval between the formation of pyrophoric carbon and the point of ignition evidently is traversed very quickly.

An observation similar to that of Ranke was made by Musselman (*6*) in the summer of 1935. Spontaneous ignition had occurred in one of three experimental stacks of chopped alfalfa hay. The temperatures varied at different points in the stack, and were excessive at some points. Within 2 weeks after stacking, the temperature had risen to 144° F. (approximately 62° C.). The temperatures then continued to rise and were maintained just below 100° C. for 40 days. A further rise occurred during the next 16 days, and then, in the succeeding 10 days, the temperature rose rapidly until ignition occurred on one side of the stack. The fire was kept under control by continued wetting for 3 weeks while observations were being made. It was noted repeatedly that the dark-brown or almost black hay would ignite when sufficiently insulated and given very little air. After the glowing surface of the interior mass had been removed until no evidence of carbonization could be seen, about a bushel of brassy-brown material was removed with the bare hands and placed upon a square piece of heavy paper, the corners of which were folded over and pinned so that the material was in a compact form but loosely enclosed to admit air.

This package lay on the ground for 5 hours without visible evidence of burning, but in 20 hours it had all been consumed.

During the summer of 1934 Roethe, Bradshaw, and Hoffman (*8*) were afforded the opportunity to investigate a barn fire in a mow containing approximately 200 tons of chopped alfalfa. The roof and upper part of the barn were destroyed before the fire was extinguished, but very little of the hay was burned. Observations made in the mass of unburned hay after the fire disclosed the presence of numerous hot spots, which were comparatively small and highly localized. The decomposed hay in these hot spots had the appearance of a black, dry mass of carbonized matter. Wherever a fire pocket had formed, there was a small central core of ash. Surrounding this carbonized hay was damp black hay that still retained its original form and had undergone only a browning on the interior of its stems. Beyond this section the hay was dark brown, dry, and glossy, and still farther toward the exterior very dry light-brown hay of dull finish appeared. The temperatures in these zones increased progressively as the inner zone was approached by removal of the hay, but the temperatures actually observed were greatly influenced by exposure of the hay during removal. Just within the surface of the black damp hay surrounding one of the hot spots a temperature of 88° C. was recorded, but smoking and finally ignition followed longer exposure.

In consideration of all the facts and views presented above, it must be concluded that hay may be made susceptible to spontaneous ignition without undergoing a decomposition approaching complete carbonization. In a limited and strictly scientific sense the term "pyrophoric" applied to hay carbon involves the assumption that such hay carbon exists in a very fine state of subdivision or has become extremely porous. In a broader sense, any substance is pyrophoric that ignites spontaneously. In the latter sense at least, the designation "pyrophoric carbon," as used by Ranke and others, would properly apply to the carbonlike material resulting from a severe carbonization of hay which is sufficiently incomplete to permit the material to retain its pyrophoric property and to ignite almost immediately upon exposure to the air. The observation of Ranke that this pyrophoric property was lost when the carbonized matter of his laboratory tests had been deprived of all its volatile empyreumatic substances has been confirmed by the writer in similar experiments. On the other hand, in the broader sense, hay that has reached a condition which falls short of the carbonaceous condition designated as pyrophoric carbon but in which it is capable of subsequent progessively rapid oxidation resulting ultimately in ignition may not correctly be called pyrophoric carbon. Such hay, however, should be referred to as pyrophoric hay, for it is indeed pyrophoric in the sense that it ignites spontaneously after sufficient oxidation.

The designation "pyrophoric hay" therefore becomes applicable to the deep-brown rowen of Ranke, the light-brown product of Truninger and the supposedly normal, properly fermented hay referred to by him, the brassy-brown material of Musselman, and the dark-brown and black products observed by Roethe, Bradshaw, and Hoffman. Furthermore, when these have been proved to be formed in the manner suggested, the products of the long-continued effect of heat or bacterial action on hay at comparatively low temperatures as predicted

y Miehe, and the unsaturated, highly unstable, intermediary fer-ıentation products formed by micro-organisms as suggested by Browne (*1*), also should be included in this class of pyrophoric ubstances.

The conception of pyrophoric hay developed in this discussion of he pyrophoric carbon theory and related explanations of spontane-us ignition leads logically to the conclusion that a pyrophoric condi-ion in hay may exist over a considerable temperature range. If this ondition is acquired at temperatures as low as that required for the roduction of brown or of black hay, hay becomes pyrophoric below he upper temperature limits of bacterial life, that is, below 80° C. ccording to Truninger, the temperature at which brown hay is formed es between 55° and 70°, while black-hay formation occurs above 70°. his conclusion supports the view of Miehe that by virtue of the long-ontinued effect of heat or bacterial action at 70° some physical or hemical change takes place that renders the hay susceptible to purely hemical oxidation and makes even more probable the explanation of Browne involving the formation of unsaturated products in the aymow.

The effect of the preliminary formation of unsaturated products ı the mow on the subsequent rise in temperature due to oxidation ould presumably be approximately the same regardless of whether hese products were produced directly by micro-organisms or indirect-y as the result of the heat of micro-organic activity. A partial oxi-dation of these substances in a given locality in the mow should in-rease the temperature and thereby accelerate their formation, and nder favorable conditions a partial or complete oxidation in that ity should promote the formation of these substances in adjacent alities in which the oxygen supply is not sufficient to destroy them s fast as they are formed. The accumulation of unsaturated sub-stances in an adjacent locality should be greatly facilitated if in that locality air is entirely or almost entirely excluded.

The results of the present investigation show conclusively that the heating of undercured alfalfa in an atmosphere of nitrogen at approxi-mately 76° to 78° C. results in an increase in the oxygen-absorption property and that the oxygen consumed in the oxidation of the preheated alfalfa is far in excess of the carbon dioxide formed. The results prove that heat from external sources, exclusive of bacterial tion, produces some change in the constituents of the alfalfa that nders it more susceptible to oxidation. Furthermore, the results strong support to the hypothesis previously proposed that the xcess consumption of oxygen is due to an oxidation of unsaturated stances produced by heat. This oxidation is analogous to the xidation of linseed or similar oils.

Although these results were obtained in laboratory experiments der conditions not strictly comparable with those prevailing in a heating haymow, it would be difficult to explain why at the same temperatures the same results should not be obtained in the mow in localities in which air is not available during the formation of the unsaturated substances and in which subsequent conditions favor their oxidation. The conclusion seems fully warranted that in a haymow in which temperatures approximately those of the laboratory experi-ments have been reached as the result of repiratory and micro-organic

processes, together with the more limited chemical reactions, the h
will undergo fundamental changes that will render it more suscepti
to oxidation, and if this heat is sufficiently prolonged, the hay v
reach a condition that may properly be called pyrophoric.

The possibility is by no means excluded that similar experime
at temperatures considerably below 76° to 78° C. would show a ter
ency to an increase in oxygen consumption as the result of heating
an inert atmosphere and that pyrophoric hay may be formed in t
haymow heating spontaneously at the lower temperatures. Be th
as it may, having once established that pyrophoric hay is produced
the mow, there remains little difficulty in explaining satisfactorily t
processes that follow and may lead to spontaneous ignition. T
influence of varying amounts of moisture in the mow, the accessibili
of air, and a number of other conditions will determine whether
not the mow will actually ignite, but these conditions will alwa
have the same significance irrespective of how the prerequisite conc
tions for the rapid rise of temperature from 70°–80° to the temperatu
of ignition are explained. For this reason a discussion of these limiti
factors need not be undertaken here.

Finally, the significance of the results of the experiment in whi
the presence of ammonia caused a great increase in the oxygen absor
tion remains to be considered briefly. The production of ammonia
hays undergoing spontaneous heating has been observed frequen
by investigators, and it is significant that alfalfa and clover, the ha
richest in nitrogenous substances, are the most susceptible to spontar
ous combustion. This production of ammonia would cause a weak
alkaline condition, which should facilitate the conversion of mo
saccharides of the hay into easily oxidizable substances, for it is w
known that very dilute alkali solutions cause some kind of rearrang
ment of the sugar molecule, resulting in the formation of numero
unsaturated compounds.

The action of strongly alkaline solutions is more pronounced. F
instance, Mathews (*4*) has shown that when a 0.4 normal solution
potassium hydroxide is allowed to stand in contact with certain
these sugars in an atmosphere of hydrogen the rearrangement
breakdown of the sugar molecule by the alkali leads to a rap
absorption of oxygen admitted to the solution.

The experiment referred to by Browne in support of his theory
spontaneous combustion, in which hydroxyacrylic acid, CHOH
CHCOOH, was formed by treating a 1-percent aqueous solution
glucose at 67° C. with 0.5 percent of completely slaked lime in a fla
from which air was excluded, also supports the view that the presen
of ammonia in the heating haymow would greatly facilitate the form
tion of unsaturated easily oxidizable substances from the reduci
sugars of the hay under the influence of heat.

SUMMARY

A laboratory investigation of the effect of heating undercure
alfalfa in an atmosphere of nitrogen at approximately 76° to 78° C
on its oxygen-absorption property is reported.

It is shown conclusively that this heating causes an increase
oxygen absorption and that the oxygen consumed in the oxidation
the preheated alfalfa is far in excess of the carbon dioxide produced.

The results prove that under the influence of heat supplied from xternal sources, with the exclusion of bacterial action, changes take lace in alfalfa that render it more susceptible to oxidation, and they rovide further indirect evidence of the formation of unsaturated ıbstances in alfalfa hay by heat and chemical action without the ıtervention of micro-organisms.

The results are discussed in their relation to the spontaneous gnition of hay. The conclusion appears to be fully warranted that in haymow in which temperatures approximating those of the laboıtory experiments have been reached as the result of respiratory nd micro-organic processes, together with the more limited chemical actions, the hay will undergo fundamental changes that will render more susceptible to oxidation, and if this heat is sufficiently pronged under favorable conditions the hay will reach a condition that ay appropriately be called pyrophoric.

The significance of the great increase in oxygen absorption caused y the presence of small quantities of ammonia is considered. The oduction of ammonia in the heating haymow, resulting in a weakly kaline condition of the hay, should facilitate the conversion of onosaccharides of the hay into unsaturated easily oxidizable com- ounds and thereby promote spontaneous ignition.

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THE GROWTH OF HALOPHILIC BACTERIA IN CONCENTRATIONS OF SODIUM CHLORIDE ABOVE THREE MOLAR [1]

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INTRODUCTION

Those bacteria that cause the reddening of salted hides and salted fish, commonly designated as halophiles, grow more luxuriantly in laboratory media as the salt concentration increases from about 3 molar to saturation. This stimulative effect of sodium chloride has been observed and reported by Clayton (*2*),[2] Robertson (*6*), Lockhead (*5*), and Stuart and James (*13*). Halophilic bacteria also have been found to prefer slightly alkaline conditions for growth, as shown by Lefevre and Round (*4*), Stather and Liebscher (*9*, *10*), and Stuart, Frey, and James (*11*). Thus, if it could be demonstrated that solutions of commercial sodium chloride tend to become more alkaline as the concentration increases above a minimum of 3 molar, it might be possible to trace this stimulative effect to the establishment thereby of a more favorable reaction for growth.

pH OF SALT SOLUTIONS

When a glass electrode was used measurements of the pH values of solutions of various lots of sodium chloride employed in making laboratory media gave no indications that they become more alkaline with increasing concentration. In fact, many of the more impure lots of salt tended to become more acid as the concentration increased, and with chemically pure sodium chloride of analytical grade the pH values remained constant at all concentrations. The only lots of salt that had a tendency to become more alkaline with increasing concentrations were certain crude, solar-evaporated salts that were decidedly alkaline at all of the concentrations studied. In table 1 some pH values for salt solutions of different concentrations, made up with boiled distilled water from five representative lots of salt, are given. All of these salts, when used in agar media, appeared to stimulate the growth of red, chromogenic, halophilic bacteria as their concentration was increased from 3 molar to saturation.

Table 1.—*pH of salt solutions from 3 to 5 molar*

Lot No.	Material	Values for molar concentrations indicated				
		3.0	3.5	4.0	4.5	5.0
		pH	*pH*	*pH*	*pH*	*pH*
1	Commercial grade (G. A. salt)	6.65	6.50	6.23	6.20	6.12
2	Commercial grade (mined salt)	6.60	6.52	6.50	6.40	6.27
3	Commercial grade (solar-evaporated salt)	8.00	8.02	8.03	8.12	8.20
4	c. p. salt (so-called)	7.72	7.52	7.42	7.31	7.15
5	c. p. salt (analytical grade)	6.92	6.92	6.92	6.92	6.92

[1] Received for publication April 26, 1940.
[2] Italic numbers in parentheses refer to Literature Cited, p. 264.

Eh MEASUREMENTS

Inasmuch as Stuart and James (*13*) have reported that relatively low Eh values appeared to favor the growth of organisms of this type, Eh measurements were made on broths containing beef extract, Bacto-peptone, Bacto-gelatin, and different molar concentrations of sodium chloride. These measurements were made both aerobically and anaerobically, by the use of a bright platinum foil electrode and a saturated calomel-potassium chloride reference half cell at 30° C. Anaerobiosis was obtained by replacing the dissolved gases in the sample with nitrogen having a purity, as determined by analysis, of 99.8 percent.

TABLE 2.—*Aerobic and anaerobic Eh measurements made on nutrient broths containing increasing concentrations of sodium chloride*

Eh measured	Values for molar concentrations of sodium chloride indicated				
	3.0	3.5	4.0	4.5	5.0
	Millivolts	*Millivolts*	*Millivolts*	*Millivolts*	*Millivolts*
Aerobically	322	314	311	304	297
Anaerobically	221	218	228	224	221

In table 2 Eh values, as calculated from measurements made both aerobically and anaerobically on a representative series of broths containing 1 percent each of Bacto-peptone and Bacto-gelatin, and 0.5 percent of Liebig's beef extract made up in 3, 3.5, 4, 4.5, and 5 molar solutions of sodium chloride are given. The pH of these broths, as determined by a glass electrode, ranged from 6.01 to 6.08.

From table 2 it would appear that nutrient broths such as described and made up with high concentrations of sodium chloride do not contain a reversible system having a strong enough effect on a bright platinum electrode to overcome the influence of dissolved molecular oxygen, since differences as great as 101 mv. due apparently to this factor alone are shown. The anaerobically measured values were much lower than those obtained aerobically and were fairly constant at all concentrations. The higher aerobically measured values showed definite decreases with increases in the sodium chloride concentrations independent of slight changes in pH. The decrease in the aerobically measured Eh values ranged from 25 to 40 mv. depending upon the salt as the concentration was increased from 3 to 5 molar, and was very uniform. Thus, the only electrometric measurement found that could be correlated with more luxuriant growth as the salt concentration was increased appeared to be the aerobically measured Eh values.

Since oxygen solubility decreases with increasing concentrations of sodium chloride (*8*), and readings with the bright platinum electrode are higher in the presence of dissolved molecular oxygen, it is probable that the gradual decrease shown in the aerobically measured Eh values with increasing concentrations of sodium chloride is the result of differences in the relative amounts of dissolved oxygen.

GROWTH OF HALOPHILIC BACTERIA

By the use of different lots of salt, a series of nutrient agars was made up to contain 1 percent each of Bacto-peptone and Bacto-gela-

concentrations of 3, 3.5, 4, 4.5, and 5 molar, respectively, and their pH and aerobically measured Eh values were determined. Sterile Petri dishes were poured with aliquots of each agar and allowed to solidify. These were then streaked with a suspension of red halophilic bacteria that had been washed with sterile 3-molar salt solution from a salt-saturated agar slant culture. One loop of inoculum was used for each streak and three streaks were made on each plate. All plates were incubated at 30° C. for 14 days and comparative growth noted. In all cases growth was more luxuriant as the salt concentration increased, irrespective of slight decreases or increases in the pH value. There was also a definite decrease in the aerobically measured Eh value as the salt concentration increased from 3 to 5 molar. In table 3 the results obtained with a typical series of agars made up with the salt, designated in table 1 as lot No. 4, are given.

TABLE 3.—*Initial pH values, aerobic Eh values and comparative bacterial growth on nutrient agars containing increasing concentrations of sodium chloride*

Sodium chloride concentration (moles)	pH	Aerobic Eh	Comparative bacterial growth [1]	Sodium chloride concentration (moles)	pH	Aerobic Eh	Comparative bacterial growth [1]
		Millivolts				*Millivolts*	
3.0	7.12	252	+	4.5	6.97	217	+++
3.5	7.11	234	+	5.0	6.92	212	+++
4.0	6.97	228	++				

[1] + = slight growth; ++ = moderate growth; +++ = heavy growth.

These results were substantiated by quantitative counting of the bacterial populations in nutrient broths buffered at definite pH levels. Nutrient broths containing 1 percent each of Bacto-peptone and Bacto-gelatin were adjusted with Sörensen's phosphate buffers (10 ml. M/15 in 50 ml. of final volume) to pH 6.5, 6.9, 7.2, and 8.2, respectively, and the sodium chloride concentration made to approximately 3.2, 3.8, 4.4, and 4.8 molar for each pH. These media were sterilized in the autoclave for 10 minutes at 15 pounds and the pH and aerobic Eh values determined. Aliquots of 20 ml. were withdrawn aseptically from each broth and transferred to sterile 30-ml. Erlenmeyer flasks and inoculated with 0.01 ml. of a 3-molar sodium chloride solution in which had been suspended the cells of red, halophilic bacteria. In this case the cultures used were *Sarcina* sp. The organisms had been washed from a salt-saturated agar slant culture. All flasks were then incubated for 14 days at 30° C.

At the conclusion of the incubation period, direct microscopic counts were made by means of a modification of the Breed technique for counting bacterial populations in raw milk. This method involves the even spreading of 0.01 ml. of broth over 1 sq. cm. of slide, drying, fixing in 90-percent methyl alcohol for 15 to 30 minutes, degreasing in xylol for 2 or 3 minutes, drying, staining in Löffler's alkaline methylene blue for 5 minutes, washing in water, decolorizing in 95-percent ethyl alcohol, drying, and counting. By fixing in 90-percent methyl alcohol followed by washing in 90-percent ethyl alcohol for prolonged intervals, it was possible to dissolve out most of the salt that had crystallized on the smear without materially affecting the count. In making counts an oil-immersion lens with 10× compensating oculars was used. The value reported is the average count per

field computed from 15 fields counted from each of 3 preparations. The results are given in table 4.

TABLE 4.—*Effect of pH, aerobic Eh, and sodium chloride concentration of nutrient broth on bacterial count after incubation at 30° C. for 14 days*

VALUES FOR MOLAR CONCENTRATION OF SODIUM CHLORIDE INDICATED

3.2			3.8			4.4			4.8		
pH	Aerobic Eh	Bacterial count per field	pH	Aerobic Eh	Bacterial count per field	pH	Aerobic Eh	Bacterial count per field	pH	Aerobic Eh	Bacterial count per field
	Millivolts	*Number*		*Millivolts*	*Number*		*Millivolts*	*Number*		*Millivolts*	*Number*
6.62	356	23	6.62	356	94	6.50	352	146	6.43	344	242
7.00	314	65	6.98	307	93	6.80	302	198	6.92	290	237
7.25	304	177	7.18	302	212	7.31	302	252	7.25	279	342
8.48	242	193	8.37	234	214	8.42	227	254	8.18	239	340

They show quantitatively at all salt concentrations the stimulating action of increasing pH. The decrease in the aerobically measured Eh values, with increasing pH at each salt concentration, appears to be caused entirely by changes in hydrogen-ion concentration. However, the decrease in the aerobic Eh values, with increasing concentrations of salt at each of the buffered pH levels, would appear to be essentially independent of changes in hydrogen-ion concentration. There is a definite increase in the bacterial count with increasing salt concentration at each of the buffered pH levels. This increase, therefore, cannot be attributed to pH change, although it can be correlated with a lowering of aerobic Eh values.

EFFECT OF OXYGEN TENSION ON GROWTH

Since it has been shown previously that the lower aerobic Eh values obtained with increasing salt concentration are caused, in all probability, by decreases in the amounts of dissolved oyxgen, it would appear that the organisms under study might possibly prefer lowered oxygen tensions for optimum growth. Because of a well-defined tendency to grow on the surface of agars and broths, these organisms have been considered heretofore as strict aerobes.

The determination of the effect of the relative oxygen tensions upon the growth of a number of red chromogenic, halophilic bacteria that had been isolated previously from salts and salted hides was, therefore, undertaken. All these organisms failed to grow on nutrient salt agar plates under strictly anaerobic conditions. On agars containing 1 percent each of peptone and gelatin, made up to a concentration of sodium chloride of 5 molar and adjusted to pH 7, no differences in growth could be observed with plates incubated in an atmosphere containing a normal amount of oyxgen as compared to those in which 40, 50, and 60 percent of the air had been replaced with carbon dioxide, nitrogen, or illuminating gas.

On agars of the same pH and organic composition made up to a concentration of sodium chloride of only 3 molar, growth appeared definitely to be stimulated when 40 and 50 percent of the air was replaced with either carbon dioxide, nitrogen, or illuminating gas.

In these experiments all plates were incubated at room temperature (24° to 28° C.) in Novy jars. The percentage of air in the atmosphere of these jars was regulated by evacuation followed by a release of the vacuum with an excess volume of gas of a previously adjusted composition.

DISCUSSION

The inclusion here of aerobic Eh measurements was prompted by the knowledge that many of the measurements reported in the past, such as those of Hewitt (*3*), were made aerobically, and that Cannan, Cohen, and Clark (*1*) in some of their earlier work found it unnecessary to exclude air in crude measurements of some reversible systems. Although the aerobic Eh measurements presented can be said to have served a purpose, the fact cannot be overlooked that the nutrient broths used did not contain a reversible system of a type that could be studied without the exclusion of air, so far as true Eh values are concerned.

In this work, however, satisfactory agreement could be secured with different bright platinum electrodes in both the aerobic and anaerobic measurements. This would indicate, according to the recent work of Ward (*14*), that the media studied did contain some type of a reversible system not precipitated by high concentrations of salt.

The effect of oxygen on the electrode appeared to be roughly proportional to the amount dissolved in the substrate. This suggested that the organisms studied might prefer, under certain conditions, reduced oxygen tension for growth. This indication was borne out by observations upon growth made in atmospheres containing reduced oxygen tensions.

Stuart and James (*12*) studied the effect of increasing salt concentrations on the Eh of protogenous media at various pH levels. They used flowing nitrogen to replace the dissolved molecular oxygen, and at concentrations above approximately 3 molar found that the Eh values remained relatively constant. The work here reported tends to substantiate this finding.

On the basis of these studies, red chromogenic, halophilic bacteria of the type causing the flesh reddening of salted hides and skins and salted fish would appear to be stimulated in their growth by slight reductions in oxygen tension. The tendency of these organisms to be strictly surface growers, therefore, must be dependent upon some factor other than a high oxygen requirement. It is suggested that this growth characteristic may be strictly a surface tension phenomenon.

A preference for a slightly reduced oxygen tension may account in part for the stimulating effect of increasing concentrations of salt on the growth of halophilic bacteria when the pH is maintained at a constant value. Rockwell and Ebertz (*7*) were the first to point out that the decreasing solubility of oxygen with increasing sodium chloride concentration might play a part in restricting bacterial growth. This is probably true of many of the ordinary bacterial types. These studies show, however, that those bacterial types capable of growing at high concentrations of salt prefer reduced oxygen tension for growth. This may be one of the reasons why sodium chloride tends to exert a selective influence on bacterial fermentation and protein decomposition.

SUMMARY

Commercial grades of sodium chloride in solution may ionize to become more acid or alkaline as the concentration increases from 3 to 5 molar. This would appear to result from impurities present, since the pH values of solutions of chemically pure sodium chloride of analytical grade remained constant at all concentrations at which it was measured.

Aerobically measured Eh values of protogenous salt media decreased as the salt concentration increased from 3 to 5 molar. Anaerobically measured Eh values were much lower than those measured aerobically and were practically constant regardless of salt concentration. This indicates that the decrease shown in the aerobic Eh values with increasing salt concentration can be attributed to differences in the relative amount of dissolved oxygen.

The stimulating effect on bacterial growth of increasing concentrations of salt above 3 molar when the pH is maintained at a constant value can be correlated with decreases in aerobically measured Eh values and, thus, with slight decreases in oxygen tension.

Growth studies made in atmospheres partly depleted of oxygen confirm the indications of the electrometric measurements in that under certain conditions halophilic bacteria prefer relatively low oxygen tension for optimum growth.

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EFFECT OF PROTEIN CONCENTRATION AND CYSTEINE ON GROWTH OF HALOPHILIC BACTERIA[1]

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INTRODUCTION

A more thorough understanding of the factors that control the growth of the bacteria designated as halophiles appears to be essential to the development of reliable laboratory culture methods. As a rule, these are the organisms that grow on salt-cured fish, meat, hides, and skins. It has been shown that they prefer for growth a relatively high pH [2] or low Eh [3] and reduced oxygen tension.[2]

Halophilic bacteria have considerable economic significance, as they cause discolorations, changes in texture and odor, and decomposition of salt-cured products.

As compared with the environment in which these organisms naturally grow, most laboratory media contain relatively low concentrations of protein. This study was undertaken primarily to determine the influence of protein concentration on the growth of a typical culture of a halophilic coccus. It has been extended to include the effect of the amino acid cysteine.

EFFECT OF PROTEIN CONCENTRATION

It has been observed repeatedly in this laboratory that protein concentration may affect materially the bacterial growth on media of high sodium chloride content. The following experiments showed that increasing the protein concentration may either stimulate or inhibit growth, depending on the concentration of sodium chloride.

To nutrient agars containing 1.5 parts of agar and 1 part of proteose peptone per 100 ml. and made up at the following molar concentrations of sodium chloride: 3, 3.5, 4, 4.5, and 5, respectively, 1, 2.5, 5, 7.5, and 10 percent of Bacto-gelatin were added. The pH of these agars was not adjusted, but electrometric measurements made with a glass electrode immediately after sterilization showed no greater variation in pH between any two than ± 0.2.

Plates were poured with each of the 25 agars and allowed to solidify. They were then inoculated by the streak method. With a platinum loop, three streaks were made on each plate from a saturated sodium chloride solution in which the cells of red chromogenic halophilic bacteria (*Sarcina* sp.) had been suspended. All plates were incubated at 30° C. for 21 days. Comparative growth, shown in table 1, is expressed by plus (+) signs. The comparative growth

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[2] STUART, L. S. THE GROWTH OF HALOPHILIC BACTERIA IN CONCENTRATIONS OF SODIUM CHLORIDE ABOVE THREE MOLAR. Jour. Agr. Res. 61: 259-265, 1940.

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may be more clearly seen perhaps in figure 1, in which a typ group of plates is shown. The plate shown in *A* was given a tl plus (+++) value, as were also *D* and *E*; *B* was given a one p (+) value; *C*, a two plus (++) value; and *F*, a four plus (+++ value.

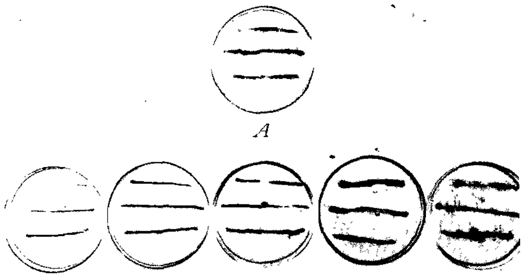

FIGURE 1.—Growth of halophilic bacteria (*Sarcina* sp.) after 21 days' incubati at 30° C. on media containing 1 percent (*A*) of Bacto-gelatin at 5-molar co centration of sodium chloride, and 1 (*B*), 2.5 (*C*), 5 (*D*), 7.5 (*E*), and 10 (percent of Bacto-gelatin at 3-molar concentration of sodium chloride.

TABLE 1.—*Growth of halophilic bacteria (Sarcina sp.) at 30° C. on media contai ing different concentrations of protein at various concentration of sodium chloride*

Concentration of sodium chloride (moles)	Growth at protein (Bacto-gelatin) concentration indicated				
	1.0 percent	2.5 percent	5.0 percent	7.5 percent	10.0 percent
3.0	+	++	+++	+++	++++
3.5	+	+++	+++	++++	++++
4.0	++	++	+++	++	++
4.5	+++	+++	+++	++	++
5.0	+++	+++	+++	++	+

The sign + indicates slight growth; ++, moderate growth; +++, heavy growth; and ++++, ve heavy growth.

The results in table 1 show that the stimulating effect of increasin concentration of sodium chloride on bacterial growth, which has bee attributed in part to lowered oxygen tension,[4] is only apparent wit media containing the lower concentrations of protein. With 5 per cent of Bacto-gelatin no differences in the growth at the differen sodium chloride concentrations could be detected. With higher con centrations of Bacto-gelatin, more luxuriant growths were obtaine at the lowest sodium chloride concentrations.

For verification of these results, counts were made of the bacteria population of nutrient broths containing 1 percent of Bacto-pepton

and 1, 3, 6, and 9 percent of Bacto-gelatin. Four concentrations of sodium chloride were used, namely, 3.2, 3.8, 4.4, and 4.8 molar, and these were buffered at the three pH levels, 6.5, 6.9, and 7.2, with Sörensen's phosphate buffers (10 ml. M/15 in 50 ml. of broth). This provided 48 different media. The pH of each medium was determined with a glass electrode, immediately after sterilization. Aliquots of 20 ml. each were then transferred to 30-ml. sterilized Ehrlenmeyer flasks and inoculated with 0.01 ml. of a saturated brine suspension of red halophilic bacteria (*Sarcina* sp.). All flasks were incubated for 14 days at 30° C. Direct microscopic bacterial counts were made by a modification of the Breed method, described in a previous communication.[5] The results are recorded in table 2.

TABLE 2.—*Bacterial count per field of halophilic bacteria (Sarcina sp.) at 30° C. in Bacto-peptone media containing different concentrations of protein and sodium chloride at various pH levels*

Concentration of sodium chloride (moles)	pH and bacterial count at protein (Bacto-gelatin) concentration indicated							
	1 percent		3 percent		6 percent		9 percent	
	pH	Count per field	pH	Count per field	pH	Count per field	pH	Count per field
3.2	6.62	23	6.40	44	6.41	133	6.53	142
	7.00	65	6.91	179	6.90	202	6.81	252
	7.25	177	7.13	186	7.15	192	7.00	282
3.8	6.62	94	6.35	96	6.40	113	6.35	111
	6.98	93	6.87	118	6.92	162	6.81	172
	7.18	212	7.17	242	7.11	208	7.00	188
4.4	6.50	146	6.52	18	6.49	12	6.35	12
	6.80	198	6.93	187	6.97	86	6.90	82
	7.31	252	7.15	287	7.05	193	7.10	188
4.8	6.43	202	6.30	12	6.43	8	6.32	0
	6.92	237	6.94	60	6.91	21	6.90	13
	7.25	242	7.20	294	7.11	28	7.16	12

Although a relatively dilute (M/15) buffer was employed in the preparation of the media, there was considerable precipitation of insoluble phosphates during sterilization. This affected the pH to an appreciable degree. In some instances, where the pH after sterilization was so changed as to be entirely out of line with the intended buffered level, it was necessary to readjust the medium with either normal sodium hydroxide or hydrochloric acid.

The results in table 2 show that with a 3.2 molar concentration of sodium chloride, the bacterial count increased at all pH levels as the protein increased. This was also true with a 3.8 molar concentration of sodium chloride at the two lower pH levels. At the highest pH level, however, increase in growth was not apparent. With 4.4 and 4.8 molar concentrations of sodium chloride, growth decreased at practically all pH levels as the protein concentration became greater.

A stimulating effect on growth was shown with an increase in the pH of the medium. With 1 percent of Bacto-gelatin the count increased as the sodium chloride concentration increased. With 3 percent of Bacto-gelatin this was only true at about pH 7.2. With

6 and 9 percent of Bacto-gelatin no stimulation of growth occurre with increasing concentrations of sodium chloride. On the contrary growth appeared to be markedly retarded.

A number of the results in table 2 suggest that increasing th concentration of protein tends to offset the unfavorable effect of lo pH. For example, with a 3.2 molar sodium chloride concentratio growth with 9 percent of Bacto-gelatin at pH 6.53 and 6.81 wa comparable to that with 1 percent at pH 7.25, whereas with 1 percen of Bacto-gelatin at pH 6.62 and pH 7.0 and with 3 percent at pH 6.40, growth was apparently retarded. Similarly, with a 3.8 mola concentration of sodium chloride, growth with 9 percent of protein a pH 6.81 was slightly greater than with 6 percent at pH 6.92. Growt with 6 percent of protein at pH 6.92 was greater than with 3 percen at pH 6.87, and growth in the latter concentration at pH 6.87 was, ir turn, greater than in 1 percent at pH 6.98. However, with 4.4 and 4.8 molar concentrations of sodium chloride, this offsetting effect was not observed. This effect of protein on the growth of bacteria at the lower sodium chloride concentrations is of special interest, since it parallels results obtained in studies on the effect of pH and protein concentration on the growth of the pneumococci, recently reported by Kelly.[6]

The inhibition of growth with increasing protein concentrations at 4.5 and 5 molar salt concentrations shown in table 1, and the lower counts with increasing protein concentrations at 4.4 and 4.8 molar salt concentrations, shown in table 2, are difficult to explain.

PROTEIN SOLUBILITY

The following results with dried egg white indicate that the solubility of protein in solutions of sodium chloride of 3 to 5 molar concentrations may vary from 50 to 100 percent. This may be one factor responsible for inhibiting growth.

Into each of a series of 50-ml. glass-stoppered graduates 10 gm. of dried egg white was placed. Sodium chloride solution of 3, 3.5, 4, 4.5, and 5 molar concentrations, respectively, were added to bring the total volume to 50 ml. After 24 hours the supernatant liquid was decanted and centrifuged. The protein nitrogen in 25 ml. aliquots of the clear centrifuged supernatant liquid is shown in table 3.

TABLE 3.—*Solubility of protein (dried egg white) at different concentrations of sodium chloride based on protein nitrogen*

Concentration of sodium chloride (moles)	Soluble protein nitrogen [1] (in 25-ml. aliquots of supernatant solution)	Concentration of sodium chloride (moles)	Soluble protein nitrogen [1] (in 25-ml. aliquots of supernatant solution)
	Gram		*Gram*
3.0	0.1328	4.5	0.1539
3.5	.1848	5.0	.1295
4.0	.2137		

[1] K. G. A. method.

[6] KELLY, WILLIAM H. THE EFFECTS OF ACIDITY UPON THE GROWTH OF PNEUMOCOCCUS IN CULTURE MEDIA CONTAINING PROTEINS. Jour. Expt. Med. 67: [illegible]

The dried egg white was more soluble in 3.5, 4, and 4.5 molar sodium chloride solutions than in 3 molar. Solubility did not increase directly, however, with increasing concentrations of sodium chloride. It reached a maximum at 4 molar and then decreased abruptly at 4.5 molar. Similar results have been obtained with casein, dried beef blood, and collagen. Thomas and Foster [7] found that the hydrolysis of collagen in sodium chloride solutions increased in the concentration range from 3 to 4 molar but decreased with greater concentration.

In these studies only one time interval was employed. The differences in solubility, therefore, may have been influenced somewhat by the rate of solubility or the rate of chemical hydrolysis. Nevertheless, it is believed that the solubility figures given are indicative of the changes brought about in the protein concentration of culture media by high concentrations of sodium chloride.

ABSORPTION OF SODIUM CHLORIDE

The removal of sodium chloride from the medium by the proteins salted out may be another factor that influences growth. That relatively large amounts of sodium chloride may be removed from solution by absorption or combination with proteins salted out at 4.5 and 5 molar concentrations of sodium chloride is clearly illustrated by the following study.

Five 1-gm. portions of collagen (hide powder), casein, and blood fibrin, respectively, were put into 50-ml. glass-stoppered volumetric graduates. This provided a series of five graduates for each protein. To each series 3, 3.5, 4, 4.5, and 5 molar sodium chloride solutions were added to bring the final volume to 25 ml. The graduates were thoroughly shaken and allowed to stand for 24 hours. Aliquots were then removed for determination of chlorides, which was done volumetrically by titration with 0.1 normal sodium sulfocyanate solution in the presence of a ferric indicator, after digestion with hot concentrated nitric acid and addition of an excess of 0.1 normal silver nitrate solution. The chlorides were calculated as sodium chloride. The results are given in table 4.

TABLE 4.—*Concentration of sodium chloride solutions before and after contact with various insoluble proteins*

Concentration of original sodium chloride solutions		Concentration after contact with the insoluble proteins—		
As made up	By titration	Collagen	Casein	Fibrin
Moles	*Moles*	*Moles*	*Moles*	*Moles*
3.0	3.02	3.03	3.00	3.05
3.5	3.43	3.39	3.43	3.39
4.0	3.93	3.89	3.93	3.93
4.5	4.46	4.13	4.32	4.27
5.0	5.18	4.13	4.39	4.39

Sodium was not determined, but pH measurements were made on the brines both at the beginning of the experiment and after they had stood for 24 hours. If the chlorine ions had been preferentially adsorbed, it seems fairly safe to assume that marked increases in pH values would have been recorded. Such was not the case.

[7] THOMAS, ARTHUR W., and FOSTER, STUART B. THE DESTRUCTIVE AND PRESERVATIVE EFFECT OF NEUTRAL SALTS UPON HIDE SUBSTANCE. Indus. and Engin. Chem. 17: 1162–1164, illus. 1925.

A marked increase in the absorption of sodium chloride by the protein products from 5 molar solutions as compared with solutions of lower molarity is clearly shown. It can be calculated from the data in table 4 that at a 5 molar concentration the absorption is equivalent to approximately 1 gm. of sodium chloride per gram of dry protein.

EFFECT OF CYSTEINE

The inclusion of small quantities of cysteine in media has aided materially in cultivating at lower sodium chloride concentrations organisms that had been isolated on sodium chloride-saturated agars. It was deemed advisable, therefore, to determine the relative effect of cysteine on growth at various sodium chloride concentrations. Nutrient agars were made up at the following molar concentrations of sodium chloride: 3, 3.5, 4, 4.5, and 5. These agars all contained 1 percent each of proteose peptone and gelatin and 2 percent of agar. Each lot of agar was then divided into three equal portions, which were adjusted with 0.1 normal hydrochloric acid and 0.1 normal sodium hydroxide to pH 6, 7, and 8, respectively, and sterilized. The adjusted portions were then each divided into two equal parts, to one of which 0.01 percent of cysteine-hydrochloride was added. The plates were poured immediately, allowed to solidify, and streaked with suspensions of test organisms in the manner described for studies of the effect of protein concentration on growth (p. 267). Control plates incubated with the test plates did not show any growth, indicating that any contaminating organisms that might have been added with the cysteine-hydrochloride would not grow on the media used. The results of these experiments are given in table 5.

TABLE 5.—*Growth of halophilic bacteria ((Sarcina sp.) at 30° C. on media at different sodium chloride concentrations and different pH levels, with and without the addition of cysteine*

Cysteine	pH	Growth at sodium chloride concentration indicated				
		3 molar	3.5 molar	4 molar	4.5 molar	5 molar
Not added	6	+	+	+	+	+
Added	6	+	+	+	+	+
Not added	7	+	++	++	+++	+++
Added	7	++++	++++	++++	+++	++
Not added	8	++	++	+++	++++	++++
Added	8	++++	++++	++++	++++	++++

The sign + indicates growth; ++, moderate growth; +++, heavy growth; ++++, very heavy growth.

The effect of adding cysteine was somewhat similar to that of increasing the protein concentration. Cysteine markedly stimulated growth with sodium chloride at molar concentrations of 3, 3.5, and 4 at pH 7 and 8. At pH 6 cysteine had no apparent effect on growth.

The macroscopic observations given in table 5 were confirmed by bacterial counts. Nutrient broths containing 1 percent of Bacto-tryptone and 1 percent of Bacto-gelatin were made up at sodium chloride molar concentrations of 3.2, 3.8, 4.4, and 4.8 and buffered at pH levels of 6.5, 6.9, and 7.2 with Sörensen's phosphate buffer. After the media were sterilized, actual pH values were determined by means of a glass electrode. Duplicate 20-ml. aliquots of each medium

were transferred aseptically to sterile 30-ml. Erlenmeyer flasks. To one of each pair of flasks 2 mg. of cysteine was added. Each flask was then inoculated with a 0.01-ml. suspension of halophilic bacteria (*Sarcina* sp.), and all flasks were incubated at 30° C. for 14 days. Direct microscopic counts were then made. The results are shown in table 6.

TABLE 6.—*Bacterial count per field of halophilic bacteria* (*Sarcina sp.*) *at 30° C. in media at different sodium chloride concentrations and different pH levels, with and without the addition of cysteine*

Cysteine	pH and bacterial count at sodium chloride concentration indicated							
	3.2 molar		3.8 molar		4.4 molar		4.8 molar	
	pH	Count	pH	Count	pH	Count	pH	Count
Not added	6.80	58	6.74	86	6.80	108	6.69	163
Added	6.71	128	6.68	133	6.69	124	6.67	151
Not added	7.11	87	7.06	93	7.09	92	7.00	196
Added	7.02	217	7.01	207	7.10	214	6.97	212
Not added	7.34	118	7.22	116	7.41	137	7.42	206
Added	7.27	324	7.34	288	7.31	311	7.26	316

In table 6, with one exception, a higher count is shown in media to which cysteine was added. At the two higher pH levels the counts were as great at sodium chloride concentrations of 3.2 and 3.8 molar as they were at 4.4 and 4.8 molar. This was not so in media without cysteine. Growth was markedly increased at the lower pH level, with sodium chloride concentrations of 3.2, 3.8, and 4.4 molar, by the addition of cysteine.

With a 3.2 molar concentration of sodium chloride, growth at pH 6.71 with added cysteine was as great as at pH 7.34 without cysteine; with 3.8 molar sodium chloride, growth at pH 6.68 with added cysteine was greater than at pH 7.22 without cysteine; with a molarity of 4.4, growth at pH 6.69 with added cysteine was comparable to that at pH 7.41 without cysteine; and with 4.8 molar sodium chloride, growth at pH 6.97 with added cysteine was comparable to that at pH 7.42 without cysteine. These results indicate that the addition of cysteine may offset slightly the unfavorable influence of low pH values, similar to the effect observed with 3.2 and 3.8 molar sodium chloride with increasing protein concentration.

DISCUSSION

Working with sodium chloride brines ranging from approximately 0 to 4 molar concentration, McLaughlin and Rockwell[8] demonstrated that increasing the concentration of soluble protein (blood) lessened the effectiveness of sodium chloride solutions in inhibiting the growth of ordinary organisms of putrefaction. In these experiments it has been shown that increasing the concentration of protein stimulates the growth of halophilic bacteria at 3 or 3.2 molar sodium chloride concentration but that this influence decreases as the concentration of brine increases to 3.8 or 4 molar. With higher concentrations of sodium

[8] MCLAUGHLIN, GEORGE D., and ROCKWELL, GEORGE E. ON THE BACTERIOLOGY OF THE CURING OF ANIMAL SKIN. Amer. Leather Chem. Assoc. Jour. 18: 233–253, illus. 1923.

chloride, increases in the concentration of protein have no stimulating effect on bacterial growth but may actually exert an inhibitory influence.

Stuart and James[9] reported that approximately 3 molar concentrations of sodium chloride were more inhibitory to halophilic bacteria than higher concentrations. Thus the stimulating effect of increasing the concentration of protein in sodium chloride solutions of 3.0, 3.2, and 3.8 molar concentration, not observed with higher sodium chloride concentrations, can be interpreted as offsetting the inhibitory action of salt. In this respect the results are in agreement with the findings of McLaughlin and Rockwell.[10]

The apparent offsetting influence of increased concentrations of protein in the substrate against unfavorably low pH values, at sodium chloride molarities of 3.2 and 3.8 paralleling similar findings for the pneumococci by Kelly,[11] suggests that this effect may hold true for most bacteria. It would require, of course, further studies on a large number of bacterial species to establish this possibility. From a practical angle it may explain many unsuccessful attempts to prevent bacterial growth through pH control in materials containing high concentrations of protein when results obtained with the usual dilute media are used as a guide.

The stimulating influence of cysteine would tend to bear out the earlier observation by Stuart and James[12] that halophilic bacteria prefer relatively low oxidation—reduction potentials for growth.

Inasmuch as this work shows that halophilic bacteria grow as well at 3 molar concentrations of sodium chloride as at higher concentrations, when small amounts of cysteine or relatively high concentrations of protein are incorporated in the medium, it would seem unnecessary to use higher concentrations of sodium chloride for their isolation and study. This would materially simplify the procedure necessary for the isolation of organisms from salt, salted meat, salted fish, or concentrated brines, for with concentrations of sodium chloride greater than 3 molar it is difficult to obtain agars clear and free from sediment.

In studies directed toward the development of more practical isolation methods for halophilic bacteria, the results reported herein have been employed to advantage. For example, agars containing 5 or 6 percent of Bacto-gelatin, a trace of cysteine, and 17.5 percent of sodium chloride with the pH adjusted to 7.2, when used for dilution plates on dirty, used sodium chloride from reddened hides and fish, have yielded counts of red, halophilic bacteria as high as a billion per gram. It is believed that agars of essentially this composition give more accurate counts of halophilic bacteria by the dilution plate method than has been possible heretofore, but further studies will be required to confirm this belief.

SUMMARY

Bacterial growth on agars, or in nutrient broths, having a concentration of sodium chloride greater than 3 molar are materially affected by protein concentration. Growth at 3, 3.2, 3.5, and 3.8 molar

[9] See footnote 3.
[10] See footnote 7.
[11] See footnote 5.
[12] See footnote 3.

concentrations of sodium chloride is stimulated by increasing the concentration of protein. At a concentration of 4 molar, growth is not appreciably affected, but at 4.4, 4.5, 4.8, and 5 molar, growth is inhibited as the concentration of protein increases.

Increasing the concentration of protein in the substrate appears to offset the influence of unfavorably low pH when the sodium chloride concentration is not greater than 3.8 molar.

The addition of small quantities of cysteine to agars and broths stimulates the growth of halophilic bacteria. There is a marked stimulation in media containing concentrations from 3 to 3.8 molar sodium chloride at pH values ranging from about 6.6 to 7.2. With higher concentrations of sodium chloride the stimulating effect of cysteine is not so noticeable.

The addition of cysteine to media also appears to offset the influence of unfavorably low pH.

The effects of the three factors—protein concentration, sodium chloride concentration, and pH—on the growth of halophilic organisms are markedly interdependent. At 3.2 and 3.8 molar sodium chloride concentrations, increasing the protein concentration causes marked stimulation of growth when the pH is low, but only slight stimulation when it is high. However, when the concentration of sodium chloride is above 3.8 molar, increasing the protein concentration tends to inhibit more strongly at the low pH than at the high. These observations re-emphasize the fact that great care must be exercised in making predictions as to the effect of changing pH and sodium chloride or protein concentration in a medium having a composition different from that of the reference medium.

FACTORS INFLUENCING LENGTH OF GESTATION AND BIRTH WEIGHT IN CATTLE[1]

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INTRODUCTION

Postnatal growth and performance in cattle are influenced to some extent by the course of prenatal development. It is important, therefore, to know what factors affect prenatal development and the degree of variation brought about by these factors. The present study was undertaken as part of a broad project to evaluate the factors that affect the efficiency of feed utilization in cattle. This paper reports only one phase of this project, the factors affecting length of gestation and birth weight.

PREVIOUS INVESTIGATIONS

Variations in birth weight and duration of gestation have been reported by Wing (*13*),[3] Eckles (*2*), Hansen (*5*), McCandlish (*10*), Fitch, McGilliard, and Drumm (*3*), Littlewood (*8*), and others. Their investigations show rather definite species and breed differences in these respects. In some cases, there was an indication that the sex of the calf influences the length of the gestation period, and it was generally concluded that there is a significant difference in birth weight between sexes.

McCandlish (*10*) concluded that length of gestation period has little influence on the birth weight of calves and that age of cow and season of calving have no influence on the duration of pregnancy. He showed, in a study of domestic cattle (*Bos taurus*), breed variations of 276 to 282 days in length of gestation period and of 49 to 94 pounds in birth weight. Eckles (*2*) indicated that calves from immature cows are smaller than those from mature cows and that cows of advanced age produce smaller calves than cows in the prime of life. Wing (*13*) reported that in 177 births, 5 sets of twins occurred which had an average gestation period of 275 days. Wing also concluded that many cows show a well-marked individual characteristic as to length of gestation period. Littlewood (*8*), in a study of Brahman cattle (*Bos indicus*), reported gestation periods of 284 to 290 days and birth weights of 41 to 62 pounds.

MATERIAL AND METHODS

During the 6-year period from 1932 to 1938, birth weights and gestation periods were obtained on 297 parturitions at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md. These included 164 parturitions from 65 beef Shorthorn cows and 133 parturitions from 68 Milking Shorthorn cows. In both herds matings were made during every month of the year so that there

[1] Received for publication June 3, 1940.
[2] Doctor Lambert resigned from the Department on March 22, 1940, to become associate director of the Agricultural Experiment Station, Purdue University, La Fayette, Ind.
[3] Italic numbers in parentheses refer to Literature Cited, p. 285.

was a fairly equal seasonal distribution of calves. All birth weights were obtained within 12 hours following birth. In calculating gestation periods, in a few cases the breeding date nearest 280 days prior to calving was used owing to the fact that some cows accepted service one or more times during pregnancy. In 6 cases out of 133 calvings in the Milking Shorthorn breed, for instance, cows accepted service during pregnancy. For example, one cow was bred 367, 337, 278, 181, and 118 days before parturition. The calf was a normal 84-pound heifer and the 278-day breeding date was used. One cow was observed to accept service during 2 out of 3 pregnancies included in this study.

In the calculation of results, all abortions were excluded, as well as one gestation period of 322 days from the last-known service. All other gestations were used, but these varied not more than 21 days above or below 280 days.

In the analyses of the data, variance and covariance analyses, as outlined by Snedecor (*11*), were used.

EXPERIMENTAL RESULTS

INFLUENCE OF SEX OF CALF ON LENGTH OF GESTATION AND ON BIRTH WEIGHT

In table 1 are shown the range in gestation period and the average birth weights by length of gestation, sex, and breed. There was about 2 days' difference between length of gestation period of the beef Shorthorn dams giving birth to bull calves and that of the dams giving birth to heifer calves. Analysis of variance showed that this difference was not highly significant, P being less than 0.05. In the Milking Shorthorns there was practically no difference between the gestation periods when they were classified according to sex of calf. This observation is in agreement with the results obtained by Knoop and Hayden (*6*), who observed differences in the Jersey but not in the Holstein breed. Littlewood (*8*) observed differences between the sexes, whereas Eckles (*2*), Fitch, McGilliard, and Drumm (*3*), McCandlish (*10*), and Wing (*13*) did not. In view of these and other observations, it is evident that length of gestation period is not greatly influenced by the sex of the calf.

TABLE 1.—*Range in gestation period of dams and average birth weights of their calves by length of gestation period, sex, and breed*

Length of gestation (days)	Average birth weights of—											
	Beef Shorthorn calves						Milking Shorthorn calves					
	Males		Females		Total		Males		Females		Total	
	Calves	Birth weight	Calves	Birth weight	Calves	Birth weight	Calves	Birth weight	Calves	Birth weight	Calves	Birth weight
	No.	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*
260–264	1	60	1	42	2	51.0	1	44	1	54	2	49.0
265–269	1	60	1	54	2	57.0	-----	-----	-----	-----	-----	-----
270–274	3	68	10	56	13	58.9	5	67	3	67	8	67.0
275–279	18	68	23	64	41	66.0	14	76	19	77	33	76.7
280–284	36	71	30	71	66	71.4	22	85	25	77	47	81.1
285–289	21	75	14	71	35	73.3	21	85	15	78	36	81.9
290–294	4	77	-----	-----	4	77.2	3	81	4	93	7	87.6
295–300	-----	-----	1	78	1	78.0	-----	-----	-----	-----	-----	-----
Total or average	84	71.5	80	66.8	164	69.2	66	81.0	67	77.5	133	79.2
Average gestation period	-----	281.8	-----	279.8	-----	280.8	-----	281.7	-----	281.6	-----	281.7

The male calves in both breeds were heavier at birth than the female calves, the average difference being 4.7 pounds in the beef Shorthorns and 3.5 pounds in the Milking Shorthorns. In the former breed the difference was found, by analysis of variance, to be highly significant, *P* being less than 0.01. In the latter breed, however, the difference was not significant, *P* being greater than 0.05. The reason for this difference between the breeds is not known but may have been due in part to the greater variability in birth weight of the Milking Shorthorn calves. In the beef Shorthorns and Milking Shorthorns, differences due to sex accounted for only 6.5 and 2.1 percent, respectively, of the total variance in birth weight.

CORRELATION BETWEEN LENGTH OF GESTATION PERIOD AND BIRTH WEIGHT

Several investigators, including Eckles (*2*) and McCandlish (*10*), have reported that length of gestation period and birth weight are not correlated. Results from the present experiment (table 1) showed that in both breeds there was a definite increase in birth weight with increasing length of gestation. An analysis of covariance between these two factors in the beef Shorthorns showed a correlation of 0.61 in the total population. When corrections were made for differences due to sex, the correlation was reduced to 0.60. In this breed, as already shown, a highly significant difference between sexes was noted for birth weight and a probably significant difference for length of gestation period. The question arises, therefore, as to whether the difference in birth weight between sexes is due to the relatively longer gestation period for the bull calves. The anaylsis of covariance indicates that 25 to 35 percent of the differences in birth weight between sexes can be attributed to differences in length of gestation period.

In the Milking Shorthorns, a correlation of 0.50 was observed between length of gestation period and birth weight in the total population, and this value remained the same when correction was made for differences due to sex. For this breed no significant differences were observed between sexes in either birth weight or length of gestation period. However, when variations in birth weight due to length of gestation period were accounted for by analysis of covariance, it was found that there was a probably significant difference between sexes, *P* being less than 0.05.

INDIVIDUAL AND BREED DIFFERENCES IN GESTATION PERIOD AND IN BIRTH WEIGHT OF CALVES

In table 2 is shown the analysis of variance of the length of gestation period between breeds and between cows. For this study, all cows with three or more gestations were selected. The average number was about four gestations per cow. The analysis shows that there is no difference between the beef and Milking Shorthorn breeds but that there is a significant amount of variation between cows. Apparently there is a tendency for a cow to have a characteristic gestation period. A cow carrying a calf a long time in one gestation is likely to do so in the next gestation. Some cows in this study had an average gestation period of 288 to 290 days, whereas others had a period of only 274 to 276 days, irrespective of the sex of the calf.

TABLE 2.—*Analysis of variance of length of gestation period*

Source of variance	Degrees of freedom	Sum of squares	Mean square
Between breeds	1	1.61	1.61
Between cows in same breed	40	1,829.16	[1] 45.73
Between gestation periods for same cow	120	2,779.26	23.16
Total	161	4,610.03	28.63

[1] Significant ($P<0.01$).

It has long been known that cows differ in the size of their calves at birth. Some cows consistently have heavier calves than do others. Breeds likewise differ to a marked degree in the average size of calves at birth. The analysis of variance of birth weights of the calves from the same cows used for the study of duration of gestation is shown in table 3. There was a highly significant difference in birth weight between the breeds as well as between cows in the same breed.

TABLE 3.—*Analysis of variance of birth weights*

Source of variance	Degrees of freedom	Sum of squares	Mean squares
Between breeds	1	2,986.93	[1] 2,986.93
Between cows in same breed	40	5,051.16	[1] 126.28
Between birth weights of calves from same cow	120	8,307.57	69.23
Total	161	16,345.66	101.53

[1] Significant ($P<0.01$).

Tables 1, 2, and 3 show the larger calves may be expected from cows having the longer gestation period. To determine the correlation between length of gestations and birth weight of calves for the same cow, a covariance analysis was made (table 4). The correlation observed in the total population was 0.50, but when differences between breeds were accounted for the correlation was raised to 0.54. The correlation between cows in the same breed definitely shows that the heavy calves were from cows whose gestation periods were longer than the mean gestation period of the breed. This correlation, 0.54, expresses the relationship between the means of birth weight and length of gestation period for each cow. The correlation between calves from the same cow does not vary greatly from that observed within breed alone. On the basis of these results, the conclusion seems justified that birth weight is influenced by length of gestation and that cows which tend to carry calves overtime, that is, longer than the mean gestation period for the breed, tend to have heavier calves than those that carry their calves undertime.

TABLE 4.—*Analysis of covariance between length of gestation and birth weight of calves*

Source of variance	Degrees of freedom	[1] SX^2	[1] SXY	[1] SY^2	Correlation coefficient	Degrees of freedom	Adjusted sum of squares	Adjusted mean square
Between breed	1	1.61	69.40	2,986.93	---	---	---	---
For test of significance of adjusted mean squares between breeds	---			---	---	1	2,858.84	[2] 2,858.84
Between cows in the same breed	40	1,829.16	1,645.35	5,051.16	0.54		---	---
Between calves from the same cow	120	2,779.26	2,620.29	8,307.57	.55	119	5,837.16	49.05
For test of significance of adjusted mean squares between cows in the same breed	-	---	---	---	---	40	3,573.21	[2] 89.33
Total	161	4,610.03	4,335.04	16,345.66	.50	160	12,269.21	76.68

[1] S=sum; X, length of gestation period; and Y, birth weight of calf.
[2] Significant ($P<0.01$).

INFLUENCE OF CALVING SEQUENCE AND WEIGHT OF DAM ON BIRTH WEIGHT AND LENGTH OF GESTATION PERIOD

In table 5 are shown the average length of gestation of the dams and the average birth weight of the calves by sequence of calving. It is obvious that there was no significant trend in length of gestation between the first and subsequent gestation periods. There was, however, some trend in birth weight. First calves were considerably lighter than subsequent calves, but beyond this point no consistent trend is evident.

TABLE 5.—*Influence of calving sequence on length of gestation of dams and on birth weight of their calves in beef and Milking Shorthorns*

Calf sequence	Beef Shorthorns			Milking Shorthorns		
	Animals	Mean *gestation* period	Mean birth weight of calves	*Animals*	Mean *gestation* period	Mean birth weight of calves
	Number	*Days*	*Pounds*	*Number*	*Days*	*Pounds*
1	44	280.0	64.3	41	281.4	74.9
2	38	282.3	70.2	39	282.1	80.3
3	28	280.7	71.7	20	282.5	84.0
4	21	281.3	71.4	13	282.7	81.9
5	16	279.2	68.7	8	279.0	77.8
6	6	278.0	69.3	6	285.2	84.2
7	3	280.7	72.3	3	279.0	77.3
8	4	283.5	76.2	2	277.0	77.0
9	3	280.3	73.3	1	283.0	70.0
10	1	285.0	78.0	---	---	---

A correlation study was made with 98 beef Shorthorns to determine the effect of length of gestation period, calving sequence, and weight of dam on birth weight of the calf. The results follow.

Correlation	*Correlation coefficient*[1]
Simple correlations between—	
Birth weight and length of gestation period	+0.55
Birth weight and calving sequence	+.21
Birth weight and weight of dam	+.22
Length of gestation period and calving sequence	−.06
Length of gestation period and weight of dam	+.00
Calving sequence and weight of dam	+.31
Multiple correlation between birth weight and length of gestation, calving sequence, and weight of dam	+.62
Partial correlations between birth weight and—	
Length of gestation, calving sequence and weight of dam constant	+.58
Calving sequence, length of gestation and weight of dam constant	+.23
Weight of dam, length of gestation and calving sequence constant	+.19

[1] +, positive correlation; −, negative correlation.

The simple correlations indicate that length of gestation had the greatest influence on birth weight of calf, whereas calving sequence and weight of dam had little influence. No correlation was observed between length of gestation period and calving sequence or between length of gestation period and weight of dam. The multiple correlation of these factors, 0.62, indicates that approximately 38 percent of the variation in birth weight of calf may be attributed to these three factors. Partial correlations indicate that length of gestation period is by far the most important of these three factors and that calving sequence is slightly more important than weight of dam.

Although the correlation between birth weight of calf and weight of dam is low, it is statistically significant. The question arises whether this correlation is due to differences between cows; in other words, whether fleshing, nutrition, and environment materially affect the birth weight of a calf from a particular cow. To determine this point, an analysis of covariance was made, the results of which are shown in table 6. The correlation between cows shows a higher relationship between mean weight of each cow and mean birth weight of calf from each cow than that observed in the whole population. The correlation between calves from the same cow (0.08) indicates that changes in weight of the cow due to environmental conditions do not materially affect the birth weight of the calf. This finding is in agreement with observations made during 1934, when cows in an extremely thin, emaciated condition as a result of the drought tended to have normal-size calves. There was no significant difference between cows in birth weight of calf when corrections were made for differences in weight of dam.

TABLE 6.—*Covariance analysis of birth weights of calves and weights of dams in beef Shorthorns*

Source of variance	Degrees of freedom	[1] SX^2	SXY[1]	[1] SY^2	Correlation coefficient	Degrees of freedom	Adjusted sums of squares	Adjusted mean square
Between cows	22	1,574,955	22,011.19	1,972.18	0.40			
Between calves from same cow	75	910,737	4,965.10	4,211.82	.08	74	4,184.76	56.55
For test of significance of adjusted mean squares between cows						22	1,706.48	[2] 77.57
Total	97	2,485,692	26,976.29	6,184.00	0.22	96	5,891.24	61.37

[1] See footnote 1 of table 4.

INFLUENCE OF SEASON OF CALVING ON LENGTH OF GESTATION AND BIRTH WEIGHT OF CALVES

Hammond (*4*) calls attention to the fact that the estrus cycle aries with the season and that there is a relationship between estrus nd length of gestation period. To determine whether season of irth affects length of gestation period and birth weight of cattle, the uration of gestation and birth weight of all calves were calculated)r each season. The results of these tabulations are shown in table 7. n beef Shorthorns the largest calves were born in the fall months, nd the longest gestation periods were observed in these months. 'he shortest gestation periods were observed in the summer months, ut the lighest calves were born in the spring months. In the Milk- ıg Shorthorns, there was little variation in length of gestation period etween seasons. The heaviest calves were born in the summer ıonths and the lightest in the spring months. An analysis of vari- nce of the effect of season shows tbat the variation between seasons as not significant.

'ABLE 7.—*Relation between season of calving and duration of gestation and birth weight*

Season	Beef Shorthorns			Milking Shorthorns			Total		
	Animals	Average gestation period	Average birth weight of calves	Animals	Average gestation period	Average birth weight of calves	Animals	Average gestation period	Average birth weight of calves
	Number	*Days*	*Pounds*	*Number*	*Days*	*Pounds*	*Number*	*Days*	*Pounds*
March to May	34	280.7	66.4	13	282.0	77.5	47	281.1	69.4
June to August	46	279.3	68.9	40	281.0	81.7	86	280.1	74.8
September to November	39	282.7	71.8	37	282.0	78.0	76	282.4	74.8
December to February	45	280.8	69.5	43	281.8	78.5	88	281.3	73 9

DISCUSSION

The findings in this study on length of gestation are in essential agreement with those reported by other investigators. The mean duration of gestation reported by others varies from 276 days for the Holstein hreed to 282 days for the Guernsey. The average duration observed in this study for the two breeds of Shorthorns is 281 days. Littlewood (*8*) found that the mean duration of gestation for *Bos indicus* cattle is somewhat longer.

Several investigators have reported that length of gestation and birth weight in cattle are not correlated. In this study these two variables were found to be correlated, the values of the correlations being 0.60 for the beef Shorthorns and 0.50 for the Milking Shorthorns. This observation is in agreement with the studies of Krasnov and Pak (*7*), who found a positive correlation between these factors. In a study of factors affecting birth weights in swine, Lush, Hetzer, and Culbertson (*9*) suggest that length of gestation has little effect except through size of litter, large litters being born at an earlier age than small litters. It seems logical that there should be a corre-

lation between these two variables since the fetus is increasing in age and should therefore be growing. Snyder (*12*) states that the length of pregnancy is subject to hormonal control and that the duration may be lengthened or shortened as desired. His observations led him to the conclusion that there may be less difference in duration between the various pregnancies of one individual than between the average duration of pregnancies of different individuals in the same race. This observation is in agreement with the findings obtained by the present authors and also with the results reported by Wing (*13*).

From the present study it would seem that birth weight is primarily an expression of the size, weight, age, and physiological constitution of the dam, and that a minor part of this variation is due to the different potentialities for growth of the calves. Therefore, birth weight of calves has a limited value as an index of their later growth potentialities. It should be noted, however, that the Arizona Agricultural Experiment Station (*1*) has observed a correlation of 0.537 ± 0.031 between birth weight and average daily gain of calves from birth to weaning. Krasnov and Pack (*7*) also observed a correlation between birth weight and adult weight of 0.56 for males and 0.41 for females. In view of these findings this problem merits further study.

SUMMARY

A study was made of the influence of the following factors on gestation period in cows and on birth weight of their calves: Sex of calf, individual differences in cows, breed, calving sequence, weight of dam, and season of calving. This study, carried on from 1932 to 1938 at Beltsville, Md., included 164 parturitions from 65 beef Shorthorn cows and 133 parturitions from 68 Milking Shorthorn cows. The average gestation period for the beef Shorthorn cows was 280.8 days and for the Milking Shorthorn 281.7 days. The respective mean birth weights of their calves were 69.2 and 79.2 pounds.

A probably significant difference between sexes in length of gestation was observed in the beef Shorthorn cows but not in the Milking Shorthorns. Differences in length of gestation period account for 25 to 35 percent of the variation in birth weight between sexes.

There is a tendency for individual cows to have a characteristic length of gestation period, and the birth weights of calves produced by any one cow tend to be less variable than those of calves from different cows. Birth weight was found to be influenced by length of gestation between calvings from the same cow, and cows which tend to carry calves overtime also tend to have heavier calves.

Breed appeared to have no effect on length of gestation, but in birth weight of calves there was a highly significant difference between breeds.

A multiple correlation indicates that the combined influence of length of gestation, calving sequence, and weight of dam account for 38 percent of the variation in birth weights in cattle. Length of gestation had the greatest effect of the three factors.

Season of calving had little influence on length of gestation or on birth weights.

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THE TOXICITY OF SARTWELLIA FLAVERIAE TO GOATS [1]

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INTRODUCTION

In March 1933 the writer was requested to investigate a serious loss of Angora goats in a flock on a ranch in Pecos County, Tex. The symptoms observed in the affected animals were a gradual loss of weight in spite of good appetite, and a distended abdomen. Autopsies revealed marked ascites and extensive hepatic cirrhosis with small calcium deposits and numerous bright yellow foci scattered throughout the cirrhotic areas. From the evidence available it was suspected that the grazing of *Sartwellia flaveriae* was the cause of the losses, and some experimental feeding tests were undertaken. The results of the investigation proved this to be a poisonous plant, but in none of the experimental animals were lesions produced similar to those that prompted the investigation. *S. flaveriae* has never been shown to be connected with livestock losses, but since the plant is frequently grazed and since it has been shown to be poisonous, conditions favorable for livestock losses from this source are possible and the results of the investigation are, therefore, reported. The plant as it occurs on the range is illustrated in figure 1.

Figure 1.—*Sartwellia flaveriae* as it occurs on the range.

DESCRIPTION OF SARTWELLIA FLAVERIAE [2]

Sartwellia flaveriae A. Gray is a yellow-flowered composite of the Helenium tribe and of the Flaveria subtribe, growing under semiarid conditions and frequently in strongly alkaline soils. It is an annual plant, branched at the base and above, 10 to 60 cm. high, frequently about 30 cm. high. The stems or branches are slender, ascending, terete or slightly angled, and glabrous. The leaves are narrowly linear, 3 to 5 cm. long and about 1 mm. wide, entire and glabrous. The numerous small heads are 12 to 17 flowered and are arranged in flat-topped cymes. The involucre is bell-shaped and 2 to 3 mm. high. The involucral bracts are in a single series, usually 5 but sometimes 6

[1] Received for publication March 23, 1940. The work herein reported was carried out in cooperation with the Bureau of Animal Industry, U. S. Department of Agriculture.

[2] Description by V. L. Cory, range botanist, Texas Agricultural Experiment Station, substation No. 14, Sonora, Tex.

in number, oval or elliptic, membranous and glabrous. The ray flowers are 3 to 5 and these have ligules 1.5 mm. long and 1 mm. broad. The disk flowers are 9 to 12 and these have a corolla 2 mm. long, the tube of which is glandular-puberulent. The achenes are 2 mm. long, 10-ribbed, almost cylindric, and hispidulous. The scales of the pappus are wholly united into a cup, which is 0.5 mm. long and has a fimbriate edge.

This species occurs in southwestern Texas and southeastern New Mexico, and particularly in flats in which the soil is more or less highly alkaline. In such habitats it may grow in pure stands. In recent years in the trans-Pecos area of Texas where sheep are being pastured this plant has markedly increased in abundance and in areas of distribution. In October 1938 it was surprising to note the occurrence and abundance of this plant below the New Mexico-Texas State line and west of the Pecos River, for in vegetation surveys made in 1927 and 1928 it was either absent or not at all prominent in most of these places.

EXPERIMENTAL METHODS

At the start of the investigation it was realized that the feeding of fresh green plant was desirable, but owing to the travel that the collection of fresh material would have required, the plan adopted was to make weekly collections of the green plant, keep part of each collection as fresh and green as possible for feeding the following week, and dry the remainder for future experimentation.

Daily forced feeding was practiced on four of the animals until death occurred, regardless of the fact that loss of appetite, the first evidence of toxicity, had been apparent for some time. The technique was later modified in order to prolong the life of the animals. According to this modified procedure, feeding was discontinued as soon as loss of appetite was observed and was not resumed until the appetite returned to normal. Feeding was then begun again and continued until this symptom reappeared. After this method had been practiced for a considerable time the feeding was discontinued for an observation period of weeks to months. Since nothing of interest developed the animals were subjected to a second feeding period. A long feeding test, therefore, consisted of a series of interrupted feeding periods, the interruptions consisting of 3- to 5-day intervals which were required for the appetite to return to normal. A total of 10 goats was employed in the investigation. The experiments were carried out at the Locoweed Laboratory at Alpine, Tex.

The nature of the plant which was fed varied according to the time of the year. The young green plant was employed in the experiments started in June, but as the season progressed the plant material grew correspondingly older until the middle of October. All tests conducted after that time were with dry material that had been collected during the summer. Most of the animals which were subjected to long and repeated feeding periods received both green and dry plant at some time during the experiment.

RESULTS OF FEEDING TESTS

The results of 3 years' experimentation with 10 goats are presented in table 1. The presentation of a detailed report on each goat seems unwarranted; therefore, in order to conserve space the total amount

of the plant which was fed during a given feeding period has been divided by the total number of days in the period in order to arrive at an average daily dose of the plant. In reality the daily dose was subject to considerable variation because vomition frequently occurred, especially about the time or immediately prior to the loss of appetite. Once vomition occurred it was useless to continue the force feeding for that particular day.

TABLE 1.—*Results of feeding Sartwellia flaveriae to goats*

Goat No.	Weight when feeding began	Dates [2] fed *S. flaveriae*	Feeding period	Total amount fed [1]	Average daily dose fed	Dose as proportion of body weight	Results
	Pounds		*Days*	*Pounds*	*Pounds*	*Percent*	
75	80	Sept. 17–Nov. 27, 1934	72	96	1.33	1.66	No ill effects; killed for autopsy Nov. 28, 1934.
82	65	Aug. 11–Sept. 4, 1934	25	20	.80	1.22	Sick Aug. 20, 1934; died Sept. 14, 1934.; liver necrosis.
103	70	Sept. 21–Oct. 7, 1937	17	26	1.53	2.18	Sick Oct. 7, 1937; died Oct. 13, 1937; liver necrosis.
90	65	Dec. 31, 1935–Mar. 3, 1936.	64	83	1.29	1.95	Sick Feb. 28, 1936; died Mar. 9, 1936; liver necrosis.
48	70	July 24–Aug. 29, 1934	37	51	1.38	1.97	Sick Aug. 14, 1934; died Sept. 3, 1934; liver necrosis.
87	55	Nov. 5, 1934–Jan. 23, 1935.	80	104	1.30	2.30	Frequent loss of appetite.
	55	June 11–Aug. 8, 1935	59	54	.91	1.65	Killed for autopsy Sept. 2, 1935.
77	75	Sept. 7, 1934–Jan. 12, 1935.	128	104	.81	1.08	No ill effects.
	90	June 11–Sept. 13, 1935	95	110	1.15	1.27	Loss of appetite.
	90	June 23–Aug. 6, 1936	45	70	1.55	1.72	Died, hydrothorax Aug. 12, 1936.
91	60	Mar. 11–May 6, 1936	57	61	1.07	1.78	No ill effects noted until Oct. 3, 1936; died hydrothorax Oct. 6, 1936.
	60	June 23–Oct. 5, 1936	105	166	1.59	2.68	
76	70	June 24–Oct. 6, 1935	105	150	1.50	2.14	Frequent loss of appetite.
	75	Sept. 9–Oct. 27, 1936	49	101	2.06	2.71	Do.
	70	Dec. 19, 1936–Jan. 22, 1937.	35	40	1.14	1.61	Died, hydrothorax Jan. 23, 1937.
95	60	Nov. 2–Dec. 19, 1936	48	100	2.08	3.46	Frequent loss of appetite.
	60	Jan. 3–Feb. 19, 1937	48	60	1.25	2.08	Do.
	60	Sept. 21–Oct. 6, 1937	16	21	1.31	2.18	Died, hydrothorax Oct. 8, 1937.

[1] Young green, old green and dry plant fed; dry plant expressed as green weight.
[2] Dates are inclusive.

Three different types of results were obtained: (1) No ill effects during the first feeding period; (2) death associated with necrosis of the liver, produced by the first feeding period; (3) death associated with pulmonary edema and hydrothorax resulting from two or more feeding periods. The negative results were obtained with goat 75 and during the first feeding period with goats 77 and 91. Of this group goat 91 received the highest daily dose, consisting of an average of 1.78 percent of the body weight and continued for 57 days with no ill effects. In contrast to these results are those with goat 82, which died after receiving 1.22-percent doses for 25 days. The apparent discrepancy may be due to the fact that goat 82 was fed the young plant, whereas goat 91 was fed plant which had been dried for several months. However, in other experiments the dry plant was found to retain much of its toxicity. The fatal results associated with necrosis of the liver were obtained with goats 48, 82, 90, and 103. In this group the feeding of the plant was continuous, regardless of the fact that a complete loss of appetite had developed and was never regained.

A comparison of the toxic content of young, old, and dry plant was obtained with this group. Goat 82 was fed the young plant, goat 103 an older stage of growth, and goat 90 a mixture of both old and young plant which had been dried for several months. From this comparison the young plant appears to be the most toxic, but both old and dry plants are seen to have retained much of the toxic principle. Death associated with pulmonary edema and hydrothorax was produced in four out of five goats which were subjected to two or more feeding period (goats 77, 91, 76, and 95). The fifth animal in this group, goat 87, was sacrificed for post-mortem studies before similar lesions may have had time to develop. The feeding of the plant was suspended in the case of these animals whenever inappetence appeared and was not resumed until the appetite returned to normal, which was generally between 2 and 5 days. After a given feeding period the animals were held for observation and if nothing of interest developed, a second feeding period was started. From the results observed in these four animals there appear to be some cumulative effects, since in the case of goat 76 frequent loss of appetite was the only ill effect observed as a result of feeding 2.14 percent doses during the first feeding period of 105 days, but in the third feeding period 1.62 percent doses caused death in 35 days. Similar results are noted for goats 77 and 95.

SYMPTOMS

The first evidence of toxicity was diminished appetite, followed within 2 or 3 days by the refusal of all food. This symptom appeared as early as the sixth and as late as the twenty-eighth day after the feeding of the plant was started. The appetite returned to normal 3 to 5 days after the feeding of the plant was discontinued. As was to be expected in the case of hydrothorax, respiration was short and rapid after the least exertion. The development of hydrothorax in the animals required but 2 to 3 days. There was a listless attitnde, but otherwise nothing of significance was noted.

PATHOLOGY

The gross lesions in the animals killed by a single feeding period consisted of an albuminous degeneration of the kidneys and numerous grayish-yellow areas scattered throughout the liver. In the cases of longer standing the thoracic cavity contained about 1,000 cc. of clear serum and all but about one-third of the base of the lungs had an edematous appearance. There was no inflammation of the lungs or pleura. The livers in the cases of long standing showed a finely mottled appearance resulting from fat changes and a slight, diffuse yellow cast which was especially noticeable on the cut surface. There was moderate evidence of cloudy swelling of the kidneys; otherwise the autopsies showed this organ to be practically normal.

Microscopically the livers of the early fatal cases showed marked focal necrosis of the parenchyma scattered at random throughout the lobule, and frequently entire lobules were involved. Between the necrotic and more or less normal liver cells the line of demarcation was usually prominent. In addition, the liver and kidney parenchyma of this type of case showed some albuminous degeneration and fatty changes but nothing approaching the extent of the changes

that were found in cases of longer standing. Pigment deposits in much of the epithelium of the liver and kidneys gave the cells a brownish-yellow cast in sections stained with haematoxylin and eosin. In the cases of longer standing there was a marked fatty infiltrative degeneration of the liver and kidney epithelium but necrosis was not a prominent part of the picture as it was in the early fatal cases. Many of the kidney tubules contained casts composed of loosely formed granular material, but actual plugging of the tubules by these casts had not occurred. There was no change in the blood vessels of the lungs which would account for the edema and hydrothorax. Phagocytosis of necrotic liver cells by polymorphonuclear leucocytes was frequently observed (pl. 1) and was especially noticeable in three cases. There was no evidence of a progressive hepatic cirrhosis; the increased amount of connective tissue observed appeared to be reparatory and there was no evidence that it was exceeding its normal function. In the preparation of specimens for paraffin infiltration liver tissues were found to contain a yellow pigment which was soluble in alcohol.

DISCUSSION

At the beginning of this investigation it was hoped that information would be obtained on the etiology of the problem which prompted the investigation as well as on the carotenosis of bovine livers as described by Buckley, Joss, Creech, and Couch.[3] The history of the goat losses suggested early carotenosis followed by hepatic cirrhosis, and as the cirrhosis developed, the loss of much of the pigment.

From the ranch where the goats unquestionably contracted the disease, the liver of a beef was obtained for study. This liver had an orange-yellow color and its microscopic appearance was similar to that of the livers described by Buckley, Joss, Creech, and Couch. *Sartwellia flaveriae* is known to occur in both Winkler and Ector Couuties in Texas, a region where a similar type of bovine hepatopathy occurs.

In considering the results of the experiments described above, it may be said that, with the exception of the necrosis and slight pigmentation of the liver parenchyma, there is nothing to suggest a fulfillment of the original objective of the work. However, before *S. flaveriae* is definitely excluded from an association with this type of pathology, tests to determine the effects of feeding young, fresh, green plant from daily collections should be made with the facilities at present available to the writer such an investigation cannot be undertaken.

In collecting the plant it was noticed that the hands of the collector acquired a yellow discoloration rather than the green of chlorophyll which is characteristic in the collection of most green plants. This was especially noticeable in the collection of the young growth. Another point of interest observed in collecting the plant was its local photodynamic properties. If the bare hands and arms were subjected to direct sunlight after handling or collecting the green plant an erythema developed, and if the exposure was of sufficient duration the reaction continued to the point of vesicle formation.

[3] Buckley, John S., Joss, E. C., Creech, O. T., and Couch, James F. Carotenosis of bovine livers associated with parenchymatous degeneration. Jour. Agr. Res. 40: 991–1005, illus. 1930.

There was no reaction when exposure to direct sunlight was prevented. This photosensitization is similar to that frequently encountered as a result of local applications of toilet waters, coal-tar derivatives, and contact with other plants.

SUMMARY

An investigation of a serious loss of goats due to hepatic cirrhosis prompted an investigation of the toxicity of *Sartwellia flaveriae*. The results of this investigation proved that this plant is poisonous. Continuous feeding of toxic doses produced death which was associated with necrosis of the liver. Long, interrupted feeding periods produced death which was associated with pulmonary edema and hydrothorax but no necrosis of the liver. Hepatic cirrhosis was not produced.

THE INHERITANCE OF AN ALBUMEN QUALITY CHARACTERISTIC OF CHICKEN EGGS[1]

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INTRODUCTION

Experimental evidence for inheritance of an egg-albumen characteristic was first adduced by Lorenz, Taylor, and Almquist (*7*),[3] who separated lines of birds that laid eggs with significantly different percentages of firm albumen. Knox and Godfrey (*4, 5*) found a significant difference between the mean percentage of firm albumen in eggs from two breeds (White Leghorns and Rhode Island Reds) and also a significantly greater variability in eggs from different hens than in eggs from the same hen. They likewise concluded that percentage of firm albumen is hereditary. Van Wagenen and Hall (*10*) presented evidence to support these findings and also demonstrated the inheritance of firm-albumen condition as estimated by comparison with photographed standards. They also found evidence of inherited differences in percentage of outer liquid albumen. Munro (*9*), using an entirely different analytical method, studied several egg-quality characteristics. He failed, however, to find that either the percentage of firm albumen or the condition of firm albumen is inherited.

The present paper records the results of 5 years' additional selection in the high and low firm-albumen lines described by Lorenz, Taylor, and Almquist (*7*), together with the results of crossing these two lines.

EXPERIMENTAL METHODS

Eggs from the various matings were incubated during the regular hatching season in March and April, and the chicks obtained were brooded with others from the Poultry Division flock. Shortly before maturity the pullets were separated from the rest and moved into laying houses equipped with trap nests. To minimize any possible effect of environmental differences, birds from all egg-quality matings were housed together. Starting about the first of each year, eggs were collected for measurement. To avoid using very small eggs, the first 10 to 15 eggs from birds that matured while measurements were being made were discarded. When possible, 10 eggs from each pullet were measured, although smaller numbers were obtained from a few birds that matured late or that paused excessively. If less than 5 eggs were obtained from any bird, those data were discarded, and the bird was eliminated from consideration.

Eggs were broken for quality measurement about 24 hours after they were laid. Since environmental temperature during the interval before measurement has an important effect on firm-albumen percentage (*8*), an attempt was made to minimize variation due to this

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[3] Italic numbers in parentheses refer to Literature Cited, p. 301.

factor by holding the eggs at 40° to 45° F., until they were broken. The percentage of firm albumen was determined by Holst and Almquist's (*3*) methods as modified by Lorenz and Almquist (*6*).

In 1933, two cockerels were chosen from the previous year's (G series) high line,[4] and one cockerel was chosen from the low line. Selected hens bred the previous year, together with sisters and half sisters of the cockerels, were mated with these males in an attempt to decrease the genetic variability of the lines in regard to percentage of firm albumen. The two high-line matings produced 89 pullets; the single low-line mating, 47. Immediately after the regular breeding season, the cockerels in the high-line matings were replaced by two cockerels obtained from a commercial poultry breeder, and 30 additional pullets were obtained from these matings.[5] All birds hatched in 1933 were banded in the H series.

In 1934–37 further selections were made, with the production of an additional 153 high-line and 111 low-line pullets. Crosses and backcrosses, in addition, produced 142 F_1 pullets, 130 backcross to high, 83 backcross to low, and 86 F_2 pullets. Beside these, in 1935, 3 of the progeny of the males from the commercial breeder were mated to 2 of the high-line males, and 21 pullets were obtained from these matings. The following year a son of one of these birds was selected to sire a mating pen. This mating, together with additional matings between progeny of the commercial breeder's males and high-line birds in 1936 and 1937, yielded an additional 40 pullets.

[4] For convenience the terms "high line" and "low line" will be used to refer to the succeeding generations of birds selected for high percentage of firm albumen and for low percentage of firm albumen, respectively.

[5] Hatchability that year was rather poor in the high line. Since conceivably an excessive amount of firm albumen might affect the embryonic development adversely, these matings were intended to distinguish between possible lowered hatchability due to the structure of the egg and that due to inbreeding. The change in males increased the hatchability enough to ascribe the excessive embryonic mortality during the regular breeding season to close inbreeding. These males were kindly donated by John E. Kimber of the Kimber Poultry Breeding Farm. The offspring of these males are referred to henceforth as the outside male line.

TABLE 1.—*Mean percentage of firm albumen for each line and cross by series and years*

Dam's line [1]	Sire's line [1]	*G series, 1932*		*H series, 1933*		J series, 1934		K series, 1935		L series, 1936		M series		Summary of all birds	
		Number of pullets	Mean percentage of firm albumen	Number of pullets	Mean percentage of firm albumen	Number of pullets	Mean percentage of firm albumen	Number of pullets	Mean percentage of firm albumen	Number of pullets	Mean percentage of firm albumen	Number of pullets	Mean percentage of firm albumen	Number of pullets	Mean percentage of firm albumen
High	High	53	66.85±0.67	89	66.80±0.41	105	67.30±0.48	41	67.63±0.80	7	67.86±1.74			295	66.63±0.28
Do	Low/high							10	63.80±1.91	17	65.71±1.12			27	65.00±1.00
Low/high	High							27	65.62± .99					27	65.52± .99
High/low	do							42	67.14± .82					42	67.14± .82
High	High/low							12	62.50± .93	16	65.75±1.63			28	64.36±1.05
Low	High					13	60.54±1.56	19	62.50±1.67	25	58.20± .91			57	60.16± .78
High	Low					35	64.14± .78	41	63.73± .67	9	63.67± .75			85	63.89± .46
Low/high	Low/high							15	03.53±1.67	21	61.67±1.19			36	02.44± .98
High/low	High/low							6	63.67±1.98	44	64.82± .90			50	63.68± .83
Do	Low							10	61.00±2.40	27	61.37± .95			37	61.27± .93
Low	Low/high							16	59.50±1.48	8	57.50±1.35			24	58.83±1.08
Do	High/low							6	61.00±2.52	16	59.75±1.03			22	60.09± .79
Do	Low	53	57.38± .80	47	53.85± .55	62	55.71± .56	26	54.69± .92	12	54.00±2.20	11	49.48±2.14	211	55.26± .37
Crosses between high and outside male lines [2]				30	65.03± .94			21	68.52± .83	26	70.31± .74	14	74.51±1.44		

[1] In the designation of dam and sire lines the female precedes the male parent. High and low refer to lines selected for high and low percentage of firm albumen. Standard errors shown.

[2] For explanation see text.

RESULTS

The results of each year's matings are recorded in table 1. Th firm-albumen percentage of eggs laid by progeny of each type of matin are averaged separately. In each of the 5 successive years, the high line pullets laid eggs with a significantly higher percentage of firr albumen than did the low-line birds. A mean of 66.63±0.28 percen firm albumen was obtained for the 295 birds in the high line during th entire period of selection. This mean includes individual birds whos eggs averaged as high as 79.3 percent, as well as some averaging as lov as 55.1. The 211 birds in the low line averaged 55.26±0.37 percent and the individual birds ranged between 39.3 and 70.7 percent firr albumen. Despite the overlapping in the distributions of the tw populations, a highly significant difference of 11.37±0.46 percen was observed over the entire period.

The significantly different firm-albumen percentage, observed for years, supports the conclusion reached previously (*7*) that this egg characteristic is inherited. Since Munro (*9*) had reached the opposite conclusion on his flock by a different method of analysis, these results were checked by duplicating his analytical method as nearly as possible.

Munro measured eggs from a group of families of sisters in a flock presumably not selected for egg-quality characteristics, and analyzed the variance of these data between and within the families. When he failed to find families significantly different for percentage of firm albumen, he concluded that the variation in this character was not, to any important degree, caused by inherited factors. The same analysis of variance was carried out on the progeny of a single year's matings of the highly selected flock described in this paper. The K series was chosen for this purpose because it contained representatives of the largest number of different crosses. Table 2 presents the results of this analysis. The highly significant F value found, 4.17, confirms the finding (presented above) that significant differences between families do occur in this flock.

TABLE 2.—*Analysis of variance of firm-albumen percentage in the K series, 1935* [1]

Source of variance	Degrees of freedom	Sum of squares	Mean square	F
Total	222	9,626.24		
Between means of dams	37	4,377.53	118.31	
Within means of dams	185	5,248.71	28.37	4.17

[1] Families of 4 or more daughters only included.
F at P=0.01 is 1.71.

In the previously published observations on these same families of birds, a significant increase in percentage of firm albumen by selection in the high line was reported; but no decrease resulted from selection in the low line. On the other hand, further selection (as reported in the present paper) failed to increase the firm-albumen percentage in the high line, although significant differences between lines were maintained consistently. In the M generation, however, the percentage of firm albumen in the low line was significantly decreased.

When, furthermore, the progeny of the high line by outside-male crosses were mated back into the high line, the group of birds obtained showed a significantly higher percentage of firm albumen than did the members of the high line.

In any attempt to evaluate the genetics of the firm-albumen percentage, the following points require consideration. This character took a large number of values between the observed extremes. First crosses were, in every case, intermediate between the high and low lines; and backcrosses were for the most part intermediate between the first crosses and the respective selected inbred lines. Judging from these observations, the multiple-gene hypothesis might be invoked to explain the mating results. At least one criterion of this mechanism is not, however, met in the present instance while another fails in part. One of these criteria requires that the variation of the F_2 generation should be greater than the variation of the F_1; the other, that the variation of the F_1 be at least no greater than that of the parent generation. According to the coefficients of variability recorded in table 3, neither of these criteria is met in full. That part of the F_1 generation which was sired by high-line males had variability greater than that of the high-line parental generation and at least as great as that of the F_2 generations.

TABLE 3.—*Coefficients of variation of percentage of firm albumen for each line and cross by series*

Dam's line [1]	Sire's line [1]	Coefficient of variation in percentage for—					
		G series	H series	J series	K series	L series	M series
High	High	7.07	5.73	7.22	7.49	6.28	--
Do	Low/high	------	------	--	9.00	6.84	
Low/high	High	-	------	------	7.69	------	------
High/low	do	--	------	------	7.81	------	------
High	High/low		------		4.91	9.73	------
Low	High			8.90	11.31	7.64	------
High	Low	------	-	7.08	6.60	3.31	------
Low/high	Low/high	------	-	------	9.85	8.63	------
High/low	High/low	------	------	------	6.95	9.13	.
Do	Low			------	11.82	7.89	------
Low	Low/high	------	------	--	9.61	9.95	------
Do	High/low	.	------	------	9.28	6.68	.
Do	Low	10.07	6.97	7.87	8.44	13.48	13.65
Crosses between high and outside male lines.[2]		------	7.66	------	5.40	6.81	6.99

[1] See footnote 1, table 1.
[2] For explanation see text.

For the most part, the percentage of firm albumen resulting from any cross approached the phenotype of the dam's line more closely than that of the sire's. The cross of low dams and high sires averaged, for example, 60.16 ± 0.78 (table 1), whereas the reciprocal cross averaged 63.89 ± 0.46, a difference of 3.73 ± 0.90 percent. These results suggest maternal inheritance. To obtain a more accurate estimate of the sires' influence, a correlation analysis was undertaken on all members of the population for which data were available for the appropriate relatives. Coefficients of correlation were calculated between the values of the daughters, the dams, the dams' sisters, and the sires' sisters (table 4). The percentage of firm albumen of the daughters' eggs was significantly correlated with that of each of these

groups of relatives. The highest correlation ($r = 0.619$) was obtaine between daughters and dams; the lowest ($r = 0.489$) between daughters and sires' sisters.

TABLE 4.—*Relative importance of the contributions of dams, dams' sisters, and sires sisters to the percentage of firm albumen of daughters* [1]

Firm-albumen percentage of daughters in relation to the firm-albumen percentage of	Coefficient of correlation	Partial beta	*P* point of partial beta	Coefficient of total determination	Coefficient of direct determination	Coefficient of join determinatio with—	
						Dams' sisters	Sires' sisters
Dams	0.6192	0.4220	<0.01	0.2613	0.1781	0.0710	0.09
Dams' sisters	.5589	.0990	0.01–.05	.0553	.0098	----	----
Sires' sisters	.4886	.2887	<.01	.1411	.0834	.0200	----
Total considered	----	----	----	.4577	.2713	[2].1864	

[1] Includes values for 901 daughers from 205 dams and 28 sires.
[2] 0.1864 is the sum of the 3 coefficients of joint determination in the 2 columns above.

The multiple correlation coefficient—using the dams, dams' sisters and sires' sisters as independent variables—was 0.6765, showing tha 45.77 percent of the variance of the daughters' egg quality wa explained by inherited factors expressed in these three groups o relatives. All three partial regression coefficients were statistically significant; those for dams and for sires' sisters were very highly so and the coefficient for dams' sisters had a value higher than tha corresponding to the commonly accepted 5-percent point. Consequently, each independent variable may be said to have contribute significantly to the total variance.

Coefficients of direct and joint determination were calculated by Tolley's method (Elliot (*1*)) in order to estimate more exactly the quantitative influence of the independent variables on the variance of the dependent. Of the total variance accounted for (table 4), more than half (27.13 percent) could be assigned to the direct effect of the independent variables; the remainder (18.64 percent) to the joint effect of the variables in pairs. Here, again, the greater part of the influence seems to come from the dams' side. More than half (26.13 percent) of the separate determination arises from the dam, and the remainder is split between the dams' sisters (5.53 percent) and the sires' sisters (14.11 percent). If, however, the sires' phenotype had been measurable, somewhat more determination could probably be assigned to the sires' side of the matings. Comparison of the direct and joint determinations supports this view. Thus most of the separate determination of the sires' sisters is direct determination (8.34 percent), whereas very little of the corresponding influence of the dams' sisters is direct (0.98 percent). Most of the determination of the dams' sisters is held jointly with the dams. Conceivably, the excess direct determination shown by the sires' sisters might really be a portion of the joint determination with the sires, if the sires' phenotype were measurable; and this discrepancy suggests the existence of an appreciable but unmeasurable direct determination due to the sire.

A more direct demonstration of the sires' influence was obtained by analyzing the variance of the progeny of the dams mated to both

high- and low-line males. The existence of a significant variance in such a population, caused only by differences between the different sires, would be definite evidence that the sires influenced the firm-albumen percentage of eggs laid by their daughters. That such a result was obtained is shown in table 5. Ten dams were available for the analysis, and these had produced altogether 62 daughters by high-line and 65 by low-line sires. Although most of these dams were high-line birds, representatives of the ow and crossed lines were also included. The variance due to differences between high- and low-line sires, isolated from the variance due to differences between individual full-sister families, was decidedly greater than the experimental error. The significance of the result is made evident when the F value (59.55) is compared with the value of F (6.90) which would be exceeded in a homogeneous population of the same number of degrees of freedom only once in a hundred times on the average. This result supports the view that the sires exert more determination than is evident in the multiple-correlation analysis. It also, consequently, disposes of the complete maternal-inheritance hypothesis as inconsistent with the data.

TABLE 5.—*Analysis of variance of firm-albumen percentage in offspring of 10 dams mated to both high- and low-line sires*

Source of variance	Degrees of freedom	Sum of squares	Mean square	F	F at P =0.01
Between means of all families	19	413.37			
Between means of sire lines	1	170.30	170.30	59.55	6.90
Between means of dams	9	125.51	13.95	4.87	2.69
Interaction	9	117.56	13.06	4.57	2.69
Experimental error	107		2.86		

The variance between dams had likewise a significant F value. Particularly interesting, however, was the statistically significant interaction between dams and sires (F=4.57, with 2.69 as the 1-percent value). Its source was a differential response of the progeny to effects of dam and sire; the families with the higher average percentage of firm albumen suffered the greater differences as a result of the different sires. Thus, besides the direct contributions of the genotypes of the sire and dam to the phenotype of the progeny, there is an additional effect of the interaction between them. This fact might suggest a complementary action of some of the genes controlling firm-albumen percentage.

DISCUSSION

An attempt to postulate a complete genetic mechanism for the inheritance of firm-albumen percentage from the available data, would probably not be profitable. Certain observations can, however, be made. In the absence of clearly defined phenotypic ratios, no simple one- or two-gene pair complex would fit the data adequately; and multiple-gene inheritance is the simplest alternative.

Two criteria of the multiple-gene hypothesis are not met by the birds studied in the present investigation, because of excessively high variability among progeny of the low-dam by high-sire crosses. These criteria are based, however, on the assumption that the parents

are essentially homozygous. Although the high-line birds were probably homozygous to a considerable degree (since 5 years of selective matings failed to increase the percentage of firm albumen in this line—table 1), the same cannot be said of the low line. The fact that in the low line the firm-albumen percentage was significantly decreased in the final generation, together with the coefficient of variability of these birds as compared with the high line (table 3), suggests that the low-line birds were considerably more heterozygous in the earlier generations than were members of the high line.

Information about the genotype of a sire is, of course, even more meager than that for a dam. The results of the correlation analysis and the analysis of variance (tables 4 and 5) show the sires' importance in determining the phenotypes of the progeny but tell nothing about the sires' genotypes. The tendency for progeny of reciprocal matings to resemble the dam's family more closely than the sire's, however, probably indicates that the sires were less homozygous, on the average, than the dams. Such a situation was rather more to be expected than the reverse if the phenotype may be assumed to be a reliable measure of the genotype, since sires had to be chosen on the basis of the performance of their sisters, whereas dams could be chosen on the basis of their own phenotypes. With such departures from the theoretical homozygosity in the genotypes of the parental generations, the observed abnormal variability might result from any of several different combinations of parental genotypes consistent with the available data. The multiple-gene hypothesis, consequently, may not be ruled out as an explanation of the mating results.

The failure of the low line to respond to selection for several generations makes dominance a tempting hypothesis, and the existence of dominance for low percentage of firm albumen was previously postulated (*7*). If any appreciable amount of dominance existed, however, the F_1 progeny should resemble the low line more nearly than the high; and this result was not obtained (table 1).

Munro's (*9*) findings, which led him to conclude that percentage of firm albumen is not inherited to any important extent, pose an interesting question in the interpretation of statistical results. Quite conceivably, intensive selection was necessary to establish differences large enough to be statistically significant in a character, the measurement of which is, by nature, not highly accurate; and, also, the normal range of genotypes in an unselected population would give phenotypic differences within the inherent standard error of the determination. Whether or not this hypothesis can explain the discordant results of the two papers, Munro's conclusion may bear examination on the basis of the theory of the use of the "null-hypotheses" (Fisher (*2*)). Such a hypothesis cannot be proved by a single experiment, but may possibly be disproved; or, in other words, the fact that genetic differences are not found in one flock does not disprove the existence of such differences in other flocks. Consequently, on the basis of the positive evidence previously published (*4*, *5*, *7*, *10*), together with its further confirmation here reported, one may conclude that percentage of firm albumen is a heritable character.

The behavior of the progeny of the outside males was especially interesting: the results suggest that additional genetic factors for high percentage of firm albumen, not present in the Poultry Division

ck, were carried by these males. In all probability, those genes r high firm-albumen percentage that are carried by the Poultry ivision flock were rather quickly isolated in fairly homozygous rm in the high line, whereas the ancestors of these birds were homo-gous for one or more additional genes for low firm-albumen per-ntage, the allels of which were carried by the introduced males.

SUMMARY

The heritable nature of firm-albumen percentage is confirmed, and me data are presented concerning the mode of inheritance of this g-quality character.

The two lines of birds established maintained an average difference ' 11.37±0.46 percent of firm albumen. Continued selection failed increase this difference until the final year of mating. Then the ercentage of firm albumen in the low line was significantly decreased. urther increase in the percentage of firm albumen was accomplished ly by bringing outside stock into the high line. Crossing the two es resulted in intermediate progeny, and backcrossing these prog-y produced offspring intermediate between the F_1 and the parental nerations. In general the progeny resembled the dam's line more osely than the sire's. The contribution of the sire was demonstrated be of substantial importance, however, by a multiple-correlation udy and by analysis of variance of the population. The existence ' a significant interaction between the genetic contributions of the re and dam was also demonstrated.

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THE CHEMICAL COMPOSITION AND APPARENT DIGESTIBILITY OF NUTRIENTS IN CRESTED WHEATGRASS HARVESTED IN THREE STAGES OF MATURITY[1]

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INTRODUCTION

Crested wheatgrass (*Agropyron cristatum* (L.) Beauv.), a winter-hardy, drought-resistant, long-lived perennial bunchgrass, is being produced extensively, and a study of its nutritive value has much immediate practical significance in spite of the fact that the individual plants are variable in height, shape of head, character of awns, size of seed, number of seed-bearing shoots, habit of root growth, and leafiness.

PLAN OF THE INVESTIGATION

The digestibility of the nutrients in the 4- and 10-inch clippings of standard crested wheatgrass was determined in both the fresh and the dried state. Samples of the forage (fig. 1), which was 36 inches high at the anther-falling stage, were studied in a similar fashion, since this by many workers is considered to be the proper stage of maturity at which to cut this grass for hay. Samples were also collected every 15 days from previously undisturbed plants to determine chemical changes in the plants during the period from early May to September.

REVIEW OF LITERATURE

Crested wheatgrass starts growth 1 to 2 weeks earlier in the spring than numerous other grasses, according to Love (*7*).[2] Its water requirement was reported by Dillman (*3*) to be 880±10 in one year and 1,024±34 the following year. It is hardier, more drought-resistant, and longer-lived than slender wheatgrass, according to Westover and his coworkers (*16*). During a 15-year experiment at the Montana Experiment Station (*10*) crested wheatgrass produced an average yield of 1 861 pounds of hay per acre, with smooth brome yielding 1,776 pounds and slender wheatgrass 1,770 pounds. A stand of crested wheatgrass may live for 15 years, according to Westover (*15*). It is easy to cure for hay at the anther-falling stage (*15*), the resulting forage being quite palatable to livestock.

Kirk (*6*) points out that crested wheatgrass becomes dormant during the hot summer, reviving with fall rains and cool weather. It is recommended for reseeding depleted range lands in the semiarid districts.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 310.

Although primarily used for pasture, crested wheatgrass produce good grade of hay, and on dry land, when so used, should be plantec rows, according to Jackman and his coworkers (*5*). It may be seed with alfalfa to reduce weed infestation of meadows, and its preser reduces danger of bloat to cattle or sheep on such pasture.

FIGURE 1.—Type of standard crested wheatgrass (*Agropyron cristatum* (L. Beauv.) fed to sheep during digestion experiments.

Crested wheatgrass was found to be very drought-resistant by Hopper and Nesbitt (*4*) in North Dakota and by Morris (*8*) in Colorado. Westover et al. (*16, p. 13*) found that in crested wheatgrass

reduced to a 15-percent moisture basis, the clippings of May 10 contained 21.01 percent of crude protein and 21.16 percent of crude fiber, but those of August 10 contained only 9.05 percent of crude protein and 25.61 percent of crude fiber.

Woodman and his coworkers (*19*) conducted digestion experiments with sheep, feeding them mixed grass clippings. In samples of grass from cuttings made at frequent intervals, 80 percent of the organic matter was digestible. This compares favorably with concentrates, the clippings being far superior to good-quality meadow hay.

At the Vermont Experiment Station (*9*) digestion trials with dairy cows fed artificially dried young grass and samples of the same grass fresh show them to be equally digestible. On the basis of 90.17 percent dry matter, the digestible-nutrient content of the dried grass was 64.37 and that of the green grass 63.36 percent.

In digestion trials with sheep, Watson and Ferguson (*14*) fed fresh pasture grass and portions of the same sample dried at 200° and at 600° C. The results indicated that drying at 200° had no depressing effect on the digestibility, but that dehydration at 600° had a markedly depressing action. Woodman, Bee, and Griffith (*17*) in trials with sheep used fresh young grass dried and pressed into cakes and found that steam or kiln drying did not affect digestibility, the results being the same as with the fresh grass.

Digestion experiments conducted by Woodman, Blunt, and Stewart (*18*) with two wether sheep showed that the organic matter of young pasture grass from April to May was 83.6 percent digestible, but in July digestibility had decreased to 74.0 percent. The digestibility of crude protein varied from 85.4 percent in early season to 76.6 percent in midseason. Early in the spring the nutritive value of pasture grass resembled that of concentrates rather than that of roughages. The percentage of calcium increased to a maximum in the dry season, falling off in the later stages. The phosphate percentage showed the opposite trend, its range, however, not being as wide.

CHEMICAL ANALYSES

Samples taken every 2 weeks from previously undisturbed plants were measured, cut at the ground line, and then air-dried for subsequent chemical study. The official methods of analysis were used (*2*). The results, computed to the fresh and water-free bases, are contained in table 1. They show rather marked trends, especially if the September 1 sample is omitted in view of the fact that it was rather dry. The crude ash, fiber, and nitrogen-free extract on a percentage basis seemed to increase as the season progressed, while the protein, decreasing rather markedly at first, remained nearly stationary after June 30. The calcium and phosphorus showed no particular changes throughout the season when compared on the fresh basis.

Crested wheatgrass cut May 3 and analyzed on a water-free basis contained 20.59 percent of protein and 21.13 percent of crude fiber. That cut September 1 contained only 3.22 percent of protein and 33.34 percent of crude fiber. The contrast between the early stages of this grass and the more mature stages is quite marked in respect to important nutrients, as shown in table 1.

TABLE 1.—*Relationship of height and seasonable development to chemical composition of crested wheatgrass*

Date sampled	Height of plant	Vegetative stage	Fresh basis: Water	Fresh basis: Crude ash	Fresh basis: Crude protein (N×6.25)	Fresh basis: Crude fiber	Fresh basis: N-free extract	Fresh basis: Ether extract	Fresh basis: Calcium	Fresh basis: Phosphorus	Water-free basis: Crude ash	Water-free basis: Crude protein (N×6.25)	Water-free basis: Crude fiber	Water-free basis: N-free extract	Water-free basis: Ether extract	Water-free basis: Calcium	Water-free basis: Phosphorus
	Inches		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
ay 3	7		79.23	1.28	6.13	6.29	14.88	1.19			4.30	20.59	21.13	49.98	4.00		
ay 17	12		68.69	2.64	4.36	7.30	16.08	.93	0.09	0.08	8.43	13.92	23.32	51.36	2.97	0.27	0.26
ay 31	22	50 percent headed	66.49	3.68	4.26	8.14	15.87	1.56	.11	.09	10.98	12.71	24.29	47.36	4.66	.32	.27
ne 15	28		65.02	2.67	2.59	10.79	18.26	.67	.07	.07	7.63	7.40	30.85	52.20	1.92	.20	.20
ne 30	35		61.20	2.81	2.76	13.57	19.21	.45	.09	.07	7.24	7.11	34.97	49.52	1.16	.22	.18
y 15	37	Seeds past water stage and filling	51.07	3.37	1.98	15.63	26.73	.62	.10	.07	6.97	4.10	32.34	55.31	1.28	.20	.15
y 30	37	Seeds in medium dough stage	45.51	4.04	2.09	17.78	29.70	.88	.12	.07	7.41	3.84	32.63	54.51	1.61	.21	.13
g. 15	36	Seeds fully mature	42.81	5.06	2.09	17.88	31.31	.85	.13	.07	8.85	3.65	31.26	54.75	1.49	.23	.14
t. 1	36	Ripe, dry	6.18	6.10	3.02	31.28	52.36	1.06	.20	.09	6.50	3.22	33.34	55.81	1.13	.21	.10

Little change in the percentages of calcium in the clippings was observed. Values of 0.27 and 0.32 percent were observed in May, after which period a value of 0.20 percent seemed to persist. Phosphorus declined gradually from values of 0.26 percent in May to 0.10 in September.

DIGESTION EXPERIMENTS

In the studies to be described the forage samples were fed during 10-day digestion experiments, in each case preceded by a preliminary feeding period of equal length. A description of the equipment and methods employed have been given fully elsewhere (*11*). Six range ewe lambs, varying in weight from 40 to 50 kilograms, so that they could be grouped into light, medium, and heavy pairs, were selected for the study. The lambs were out of Lincoln × Merino ewes and sired by Hampshire rams. The condition and maturity of the clippings are placed in the following five groups:

1. Fresh 4-inch clippings.
2. Dried 4-inch clippings.
3. Fresh 10-inch clippings.
4. Dried 10-inch clippings.
5. Dried clippings at anther-falling stage (hay).

The samples of feed were thoroughly mixed. The forages were fed at the same level of dry-matter intake, although some variation in the case of sole roughage feeding would not be a seriously disturbing factor (*1*).

The composition of the forages fed to the lambs is shown in table 2. Since a 2-inch aftermath was left in making these clippings, the immature plants were actually 6 and 12 inches high. The plants at the anther-falling stage were 36 inches tall before cutting. These samples should not be confused with those taken every 15 days for chemical studies only, since the latter series of samples was cut at the ground line for the purpose of studying the composition of the entire plant. Early in the spring before growth started, old grass was clipped from those areas which produced the grass for the digestion experiments. An air-circulation drier operating at a temperature of 60° C. was used to dry the clippings.

TABLE 2.—*Chemical composition of fresh and dry samples of crested wheatgrass at different stages of development*

Description of sample	Dry matter	Crude protein (N×6.25)	Crude fiber	N-free extract	Ether extract	Ash
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Fresh 4-inch clippings	32.40	4.43	8.19	15.81	0.92	3.05
Dried 4-inch clippings	88.87	13.69	19.83	43.82	3.11	8.42
Fresh 10-inch clippings	36.58	3.02	10.27	19.60	.80	2.89
Dried 10-inch clippings	93.45	7.95	27.73	48.90	2.22	6.65
Dried clippings at anther-falling stage	89.81	4.80	33.98	43.88	1.49	5.66

The detailed data for the 30 individual digestion experiments which comprise this study have been omitted and averages only presented.

A summary of the coefficients of apparent digestibility is contained in table 3. The average coefficients for each trial have been computed on the basis of six lambs, and these have been used in determin-

The nutrients in the fresh 4-inch samples were all well digested to an extent similar to concentrates. When a portion of these same clippings was dried artificially at 60° C. and fed to the same six lambs, a lower digestibility of the nutrients was observed. In the case of dry matter the coefficients declined from 66.67 to 61.00. For protein the decline in digestibility was marked, being reduced from 74.33 to 65.00. The coefficients of digestibility of fiber decreased only slightly from 63.50 to 60.00, and those of ether extract from 65.67 to 59.00. The digestibility of nitrogen-free extract was reduced from 74.50 in the fresh young grass to 71.67 in the dried grass.

TABLE 3.—*Coefficients of apparent digestibility of crested wheatgrass when cut at different stages of development and fed to ewe lambs*

Description of sample fed and lamb No.	Dry matter	Crude protein (N×6.25)	Crude fiber	N-free extract	Ether extract
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
Fresh 4-inch clippings:					
7	66.00	72.00	65.00	74.00	67.00
2	63.00	78.00	57.00	70.00	72.00
3	67.00	73.00	65.00	74.00	66.00
4	71.00	77.00	68.00	79.00	67.00
5	64.00	71.00	60.00	73.00	58.00
6	69.00	75.00	66.00	77.00	64.00
Average	66.67	74.33	63.50	74.50	65.67
Dried 4-inch clippings:					
7	61.00	64.00	59.00	72.00	60.00
2	63.00	67.00	62.00	73.00	62.00
3	59.00	64.00	58.00	70.00	56.00
4	62.00	67.00	60.00	72.00	62.00
5	62.00	65.00	61.00	73.00	55.00
6	59.00	63.00	60.00	70.00	59.00
Average	61.00	65.00	60.00	71.67	59.00
Fresh 10-inch clippings:					
7	63.00	56.00	64.00	72.00	50.00
2	69.00	62.00	71.00	77.00	51.00
3	67.00	61.00	69.00	75.00	52.00
4	67.00	59.00	67.00	75.00	55.00
5	66.00	60.00	67.00	74.00	52.00
6	68.00	60.00	69.00	76.00	58.00
Average	66.67	59.67	67.83	74.83	53.00
Dried 10-inch clippings:					
7	61.00	47.00	65.00	68.00	56.00
2	67.00	53.00	75.00	73.00	60.00
3	61.00	45.00	66.00	69.00	54.00
4	65.00	49.00	70.00	72.00	59.00
5	62.00	50.00	67.00	70.00	56.00
6	62.00	44.00	68.00	70.00	57.00
Average	63.00	48.00	68.50	70.33	57.00
Dried clippings at anther-falling stage:					
7	49.00	34.00	53.00	54.00	16.00
2	44.00	34.00	48.00	50.00	8.00
3	46.00	36.00	49.00	52.00	12.00
4	48.00	32.00	50.00	54.00	24.00
5	49.00	40.00	52.00	54.00	30.00
6	51.00	42.00	53.00	56.00	32.00
Average	47.83	36.33	50.83	53.33	20.33

The decrease in digestibility as a result of drying is not so marked in the more mature grass taken at a 10-inch height as at the 4-inch height. With the exception of the protein coefficient, the other constituents showed no significant differences in digestibility of the fresh and dry clippings.

The nutrients of the 4-inch clippings were found to be better digested than those in plants 10 inches high.

The digestion coefficients of the nutrients in the sample harvested at the anther-falling stage are strikingly lower than those of the nutrients in the younger grass with the exception of nitrogen-free extract. A value of 36.33 for the protein in the older grass as compared with 74.33 for the protein in the young fresh grass is an example.

In the study of immature stages, height measurements were used as a measure of maturity. Such a procedure is open to criticism in that height alone is not a safe index of plant maturity in grasses. During periods of drought a grass 4 inches tall may be more mature from a vegetative standpoint than one twice that height during more favorable seasons. The dry-matter content of the immature grasses may also be used as an index of maturity.

The digestible nutrients based on average coefficients in table 3 are summarized in table 4.

TABLE 4.—*Digestible nutrients in the fresh and dry forage clippings of crested wheatgrass harvested at different stages of development*

Description of sample	Total dry matter	Digestible nutrients						
		Dry matter	Crude protein (N×6.25)	Crude fiber	N-free extract	Ether extract	Total digestible nutrients	Nutritive ratio *1 to —*
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Fresh 4-inch clippings	32.40	21.60	3.29	5.20	11.78	0.60	21.62	5.57
Dried 4-inch clippings	88.87	54.21	8.90	11.90	31.42	1.83	56.34	5.33
Fresh 10-inch clippings	36.58	24.40	1.80	6.96	14.66	.42	24.36	12.53
Dried 10-inch clippings	93.45	58.87	3.82	19.00	34.38	1.26	60.04	14.72
Dried clippings at anther-falling stage	89.81	42.96	1.74	17.27	23.40	.30	43.08	23.76

The 4-inch clippings with a moisture content of 67.60 percent contained 3.29 percent of digestible protein and 21.62 percent of total digestible nutrients. By drying the grass to a moisture content of 11.13 percent, the digestible protein was increased to 8.90 percent and the total digestible nutrients to 56.34 percent. This percentage of digestible protein is equal to that of irrigated alfalfa hay, and the total digestible nutrients are 16 percent higher than those found by the author (*12*) in good alfalfa hay.

The 10-inch clippings with a moisture content of 63.42 percent contained 1.80 percent of digestible protein and 24.36 percent of total digestible nutrients. When the moisture was reduced to 6.55 percent, the dried clippings contained 3.82 percent of protein and 60.04 percent of total digestible nutrients. Although low in digestible protein, the total digestible nutrient value of this dry forage is identical with that found in light-weight oats.

The anther-falling stage of crested wheatgrass, considered by many to be the ideal hay stage, produced forage having a moisture content of 10.19 percent, 1.74 percent of digestible protein, and 43.08 percent of total digestible nutrients. Thus the digestible nutrients were found to be equal to the digestible nutrients in the milk stage of Albit wheat hay (*13*). The grass hay was lower in protein than Albit wheat hay.

If the composition of the fresh clippings at the 4- and 10-inch height and at the anther-falling stage is computed on a water-free basis, an the digestible nutrients compared, the actual changes which have take place in the dry matter as the plate matured may be observed. Tabl 5 contains the data.

TABLE 5.—*Digestible nutrients in the dry matter of crested wheatgrass harvested a different stages of development*

Description of sample	Crude protein (N×6.25)	Crude fiber	N-free extract	Ether extract	Total digestible nutrients	Nutritive ratio 1 to —
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	
Fresh 4-inch clippings	10.15	16.05	36.36	1.85	66.73	5.5
Fresh 10-inch clippings	4.93	19.05	40.09	1.16	66.68	12.5
Dry 36-inch clippings at anther-falling stage	1.94	19.23	26.06	.34	48.60	23.7

When the plants were 4 inches high, the dry matter of crested wheatgrass was comparable in digestible nutrients to that of alfalfa leaves (*12*). At the 10-inch stage the percent of digestible nutrients was as high as at the 4-inch stage, but the relationship of the nutrients was different. For instance, the digestible protein decreased from 10.15 to 4.93 percent, and the nitrogen-free extract increased from 36.36 to 40.09 percent. Although the dry matter at the more mature stage was as digestible, it contained more carbohydrates. At the anther-falling stage this forage contained as high a percentage of digestible nutrients as Markton oat hay cut at the dough stage (*13*) but only 37 percent as much digestible protein.

SUMMARY

The dry matter of crested wheatgrass contained 20.59 percent of crude protein in early May, but by September the percentage had decreased to 3.22. Crude fiber and nitrogen-free extract tended to increase as the season advanced. The percentage of phosphorus decreased gradually from May to September, while calcium declined rapidly in May but remained constant at about 0.20 percent the remainder of the season. In the immature stages crested wheatgrass is an excellent forage but loses much of its nutritive value as it matures.

The protein of the fresh 4-inch clippings was 74.33 percent digestible, whereas in fresh 10-inch clippings its digestibility was only 59.67 percent.

The digestibility of all the nutrients except nitrogen-free extract was decreased by the drying process in the 4-inch clippings, but was little influenced by the drying process in the 10-inch clippings.

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FACTORS INFLUENCING THE CARBON METABOLISM OF THE CROWN GALL ORGANISM [1]

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INTRODUCTION

The large amount of unknown metabolic products and the variability of their appearance seem important for understanding the activity of the crown gall bacteria, *Phytomonas tumefaciens* (Smith and Town.) Bergey et al. For example, Conner et al [2] found that on a carbon basis about 2 percent of the fermented sugar went to carbon dioxide, 11 to 18 percent became gum and bacterial cells, while 80 to 87 percent became unidentified products. The amount of this unknown material has been found to vary enormously depending on the constituents of the medium and the conditions of fermentation.

If the nature of these unknown products were to be studied it obviously would be necessary first to determine (1) the important factors responsible for the variation in their occurrence, (2) the optimum conditions for their production, and thus (3) the experimental means for producing similar results in successive trials. Such studies are reported in the following pages.

EXPERIMENTAL WORK

CULTURE AND MEDIUM

The culture, *Phytomonas tumefaciens* A–6, was the progeny of a single cell. [3] Stock cultures were carried on yeast-water-mannitol-mineral-salts agar held at pH 7 with phosphate buffer. Pathogenicity of the culture was checked at intervals during the investigation by inoculation of tomato plants, and its purity after fermentation was checked by plating.

A synthetic medium developed by J. M. Lanen, I. L. Baldwin, and A. J. Riker was used. This medium had the following composition: Sucrose, 20 gm.; glutamic acid, 2.5 gm.; ammonium sulfate, 7 gm.; salt mixture (2 gm. $MgSO_4.7H_2O$, 2 gm. K_2HPO_4, 2 gm. NaCl, 1 gm. $CaCl_2$), 0.58 gm.; 20-percent phosphate buffer pH 7, 50 cc.; distilled water to make 1 liter. In making up the medium the ammonium sulfate, glutamic acid, and salt mixture were dissolved and the solution neutralized with sodium hydroxide. This solution, the

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phosphate buffer and the sugar in solution were sterilized separately, and then combined.

A satisfactory parent culture was a shallow liquid culture between 18 and 36 hours old, not mechanically aerated. Uniformity in transfer was assured by using equal quantities of this liquid.

All fermentations were run at 26 (±0.5)° C.

Results here reported were chosen as representative from at least three separate experiments.

AERATION METHODS

Three methods of mechanical aeration were employed. (1) A stream of sterile air was passed through Jena gas-distribution tubes (No. 33cG.4) at the bottom of 3-liter cultures in 4-liter pyrex serum jars. The gas-distribution tubes broke the stream of air into very small bubbles which arose through the culture medium with a stirring as well as an aerating effect. The air was sterilized by passage through a sterile cotton filter. Flowmeters were used to measure the flow of air through each culture. Air was passed through replicate cultures at the same rate, approximately 0.016 cubic feet per minute. (2) Method No. 1 was carried out under pressures greater than atmospheric pressure. This was accomplished by passing the air leaving each culture through a column of mercury 450 mm. high. (3) Shallow liquid cultures were agitated in a mechanical shaker, and cotton plugs were replaced by two layers of six-ply oil-treated air-filter tissue to provide more uniform aeration.

ANALYTICAL METHODS

The amount of sugar before and after fermentation was determined, after inversion, by the method of Stiles, Peterson, and Fred.[4]

Total carbon in each culture was determined by the wet-combustion method of Heck.[5] Since carbon dioxide was the only appreciable volatile metabolic product, as shown by Conner et al.[6] and confirmed by the writers, the amount of carbon dioxide produced was determined by difference between the total carbon in the culture at the beginning and at the end of the fermentation.

The gum and cells in an aliquot of culture were precipitated by the addition of four volumes of 95-percent alcohol. After several hours the insoluble material was concentrated in a centrifuge, washed three times with 80-percent alcohol, and dried in a vacuum desiccator. The dry gum and cells were washed quantitatively from the centrifuge tube and analyzed for carbon by the wet-combustion method.

The amount of fermented sugar accounted for by the known and unknown metabolites is expressed on a carbon basis. In an aerobic fermentation oxygen from the atmosphere is used and is considered as part of the substrate. The quantity of oxygen utilized is of course unknown, but by expressing sugar consumed and products formed on a carbon basis, the effect of the oxygen can be eliminated and an accurate balance can be obtained.

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[6] CONNER, RIKER, and PETERSON. See footnote 2.

FACTORS INFLUENCING THE RATE OF SUGAR FERMENTATION

The effects of aeration, amount of sugar present, size of inoculum, and addition of plant extracts on sugar fermentation are considered in order.

Comparisons were made between cultures treated (1) with no aeration, (2) with a stream of air, and (3) with a stream of air under pressure. The results are given in table 1. Likewise comparisons were made between shallow cultures shaken and unshaken, and between shaken cultures having different depths. These results are shown in table 2. In both cases with greater aeration more sugar was fermented.

TABLE 1.—*Effect of aeration on the fermentation of sugar* [1]

Aeration	Depth of liquid	Replicates	Sugar fermented per 100 cc.	
			Range	Average
	Inches	*Number*	*Grams*	*Grams*
None	1	3	0.20–0.25	0.22
Stream of air	6	4	.44– .67	.58
Stream of air under pressure [2]	6	3	1.00–1.16	1.07

[1] All cultures were allowed to ferment 4 days.
[2] 450 mm. of mercury added pressure.

TABLE 2.—*Effect of shaking and depth of liquid layer on fermentation of sugar* [1]

Aeration	Depth of liquid	Sugar fermented per 100 cc. in—		
		Replicate		Average
		No. 1	No. 2	
	Inches	*Grams*	*Grams*	*Grams*
None	½	0.26	0.22	0.24
Mechanical shaking	½	1.08	1.04	1.06
Do	¼	1.42	1.38	1.40

[1] These cultures were in 500-cc. Erlenmeyer flasks.

In certain cases the amount of sugar fermented was affected by the concentration of sugar in the medium. In cultures aerated with a stream of air more sugar was fermented in a medium with 2 percent of sugar than in a medium with 1 percent of sugar as shown by table 3. When cultures were aerated by shaking, this effect was not obtained. In cultures containing 1, 2, and 3 percent of sugar, the average amounts fermented in 4 days were 0.74, 0.71, and 0.72 gm. per 100 cc., respectively (not shown in tables).

TABLE 3.—*Effect of sugar concentration on fermentation of sugar* [1]

Original sugar concentration	Replicates	Days fermented	Sugar fermented per 100 cc.	
			Range	Average
	Number	*Number*	*Grams*	*Grams*
1 percent	4	4	0.58–0.65	0.62
		7	.74– .81	.77
		10	.97– .99	.98
2 percent	4	4	.73–1.08	.86
		7	.97–1.34	1.18
		10	1.40–1.60	1.48

[1] These cultures received a 2-percent inoculum all from the same source and were aerated under an added pressure of 450 mm. of mercury.

To a certain extent the amount of sugar fermented was increased by an increase in size of inoculum. A 5-percent inoculum usually gave the maximum sugar fermentation, but this effect was not always dependable. With cultures aerated by shaking, a 1-percent inoculum proved inferior to a 5-percent inoculum in about 75 percent of the cases (not shown in tables). Use of the larger inoculum increased the amount of sugar fermented by an average of about 20 percent.

Although the fermentation was fairly rapid in the aerated synthetic medium, it could be increased by the addition of certain plant extracts (table 4). Tomato, carrot, and yeast extracts were tried, but doubtless other extracts would also stimulated the growth. This suggests that the organism cannot synthesize its own growth factors rapidly enough for optimum growth under the conditions described.

TABLE 4.—*Effect of plant extracts on fermentation of sugar* [1]

Tissue extracted	Dry tissue represented by extract per 100 cc. of medium	Sugar fermented per 100 cc. in—		
		Replicate		Average
		No. 1	*No. 2*	
	Grams	*Grams*	*Grams*	*Grams*
Carrot [2]	0.78	2.05	2.22	2.14
Tomato fruit [2]	.58	2.16	2.39	2.28
Yeast	.40	1.96	1.92	1.94
None		1.01	1.05	1.03

[1] These cultures had a liquid depth of ½ inch, were aerated by shaking in 500-cc. Erlenmeyer flasks, and received a 5-percent inoculum.

[2] In cultures containing tomato and carrot extracts there was more than 2 percent of sugar since some sugar was present in the extracts.

UNIFORMITY AMONG REPLICATE CULTURES

Table 1 shows that replicate cultures aerated by a stream of air varied considerably in the amount of sugar fermented. Better agreement could be obtained (1) by letting the cultures ferment 10 days instead of 4, (2) by increasing the size of inoculum to 5 percent or more, and (3) by aeration under added pressure. However, the variability was reduced to an acceptable degree only when the cultures were aerated by shaking. This is shown by a comparison of the variation among replicates in tables 1 and 2.

The difference in uniformity of fermentation obtained with the two types of aeration might be explained on a basis of uniformity of aeration conditions. Cultures in flasks of uniform size and shape, agitated simultaneously in the same shaker, and covered with the same thickness of air-filter fabric were probably aerated more uniformly than cultures aerated by a stream of air passed through a gas-distribution tube. Air was passed through replicate cultures at approximately the same rate, but the dispersion of air by different gas-distribution tubes was not uniform.

DISTRIBUTION OF METABOLIC PRODUCTS

Cultures aerated by a stream of air showed variability in distribution of metabolic products as well as in the amount of sugar fermented. The amount of carbon in gum and cells was fairly uniform among replicates, but the amount of carbon in carbon dioxide and unidentified products was extremely variable. Carbon of carbon dioxide accounted for 90 percent of the fermented sugar in one culture and only 40 percent in a replicate. Since the percentage of fermented sugar accounted for as gum and cells remained fairly constant, a variation in the amount of carbon dioxide produced would mean an inverse variation in the amount of unidentified products. This irregularity, too, was partly eliminated by aerating under pressure, increasing the size of inoculum, and extending the fermentation period. But again satisfactory uniformity among replicate cultures was obtained only when the cultures were aerated by shaking, as shown by table 5.

TABLE 5.—*Effect of aeration by shaking, pressure aeration, sugar concentration in the medium, and length of fermentation on distribution of metabolic products*

Conditions	Replicate No.	Sugar fermented per 100 cc.	Carbon in fermented sugar—			
			Accounted for as—			Not accounted for
			Carbon dioxide	Cells and gum	Total	
		Grams	*Percent*	*Percent*	*Percent*	*Percent*
Liquid 1 inch deep in 9-liter serum jar aerated by shaking 4 days.	1	0.52	46	33.0	79.0	21.0
	2	.54	44.3	26.4	70.7	29.3
	3	.52	46.6	28.0	74.6	25.4
	4	.59	41.1	22.3	63.4	36.6
	Average	0.54	44.5	27.4	71.9	28.1
Liquid 1 inch deep in 9-liter serum jar aerated by shaking 10 days.	1	0.96	61.8	16.5	78.3	21.7
	1	.92	65.7	15.0	80.7	19.3
	3	.93	66.2	14.2	80.4	19.6
	4	.94	65.2	14.7	79.9	20.1
	Average	0.94	64.7	15.1	79.8	20.2
1 percent of sugar aerated 4 days [1]	1	0.58	25.2	31.0	56.2	43.8
	2	.65	43.0	25.5	68.5	31.5
	3	.61	57.2	29.1	86.3	13.7
	4	.65	71.2	30.7	101.9	−1.9
	Average	0.62	49.2	29.1	78.3	21.7
1 percent of sugar aerated 10 days [1]	1	0.99	86.0	15.7	101.7	−1.7
	2	.98	80.3	20.2	100.5	−.5
	3	.99	81.5	22.2	103.7	−3.7
	4	.97	78.0	17.1	95.1	4.9
	Average	0.98	81.5	18.8	100.3	−0.3

[illegible]

TABLE 5.—*Effect of aeration by shaking, pressure aeration, sugar concentration in the medium, and length of fermentation on distribution of metabolic products*—Con.

Conditions	Replicate No.	Sugar fermented per 100 cc.	Carbon in fermented sugar—			
			Accounted for as—			Not accounted for
			Carbon dioxide	Cells and gum	Total	
		Grams	*Percent*	*Percent*	*Percent*	*Percent*
2 percent of sugar aerated 4 days [1]	1	1.08	54.7	18.6	73.3	26.7
	2	.84	85.0	21.8	106.8	−6.8
	3	.80	60.5	20.4	80.9	19.1
	4	.73	47.2	19.7	66.9	33.1
	Average	0.86	61.9	20.1	82.0	18.0
2 percent of sugar aerated 10 days [1]	1	1.50	75.3	12.4	87.7	12.3
	2	1.60	77.3	12.3	89.6	10.4
	3	1.43	72.0	13.4	85.4	14.6
	4	1.40	62.5	13.5	76.0	24.0
	Average	1.48	71.8	12.9	84.7	15.3

[1] Cultures were aerated with a stream of air under 450 mm. of mercury added pressure. The inoculum was 1.7 percent. There were three liters of culture in 4-liter serum jars.

RATIO OF PRODUCTS

On a carbon basis, carbon dioxide usually accounted for 45 to 80 percent of the fermented sugar, while gum and cells accounted for about 15 to 30 percent. The percentage of fermented sugar unaccounted for averaged from almost none to 28 percent. In a few cases where cultures were aerated with a stream of air the yield of carbon dioxide was low and unaccounted-for sugar was as much as 50 percent. Such fermentations were not reproduced easily. In some instances a little more than 100 percent of the fermented sugar was accounted for by carbon dioxide, gum, and cells. This excess probably came from glutamic acid, since the calculations did not consider its utilization. Of course, all figures may be too low for this same reason

The percentage of fermented sugar accounted for by carbon in gum and cells was found to decrease with age of culture, while the percentage of fermented sugar accounted for by carbon dioxide increased with age of culture. The amount of unfermented sugar in the medium seemed to affect the amount of carbon dioxide produced. As a rule the percentage of fermented sugar accounted for as carbon dioxide was lower where the unfermented sugar was more abundant.

From the available data on the distribution of metabolic products it appeared that a great part of the unidentified products might be transformed readily to carbon dioxide. The presence of a considerable amount of unfermented sugar seemed to have a sparing action on the unidentified matérial and it accumulated to a certain extent. When the amount of unfermented sugar was low, very little unidentified material was found. These points are illustrated by table 5.

SUMMARY

Conditions were established whereby crown gall bacteria could be grown rapidly in a synthetic medium with only small variation in the products from carbon metabolism.

The rate of growth and sugar fermentation of the organism was considerably increased by aeration of the medium. The fermentation was further stimulated by the addition of plant extracts. The size of inoculum, the amount of aeration and, where cultures were aerated with a stream of air, the amount of sugar in the medium influenced the amount of sugar fermented.

The uniformity among replicate cultures with respect to sugar fermented and to distribution of metabolic products was influenced by the size of inoculum, length of fermentation period, and type of aeration. Aeration seemed to be the most important factor. Consistently reproducible fermentations were obtained when the cultures received a 5-percent inoculum of 24-hour liquid culture and were aerated by shakiug.

The ratio of metabolic products was related to the concentration of unfermented sugar in the medium. When the concentration of unfermented sugar was high, the ratio of carbon dioxide to unidentified products was low and when the concentration of unfermented sugar was low, the ratio of carbon dioxide to unidentified products was high. This ratio increased with age of culture.

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EFFECTS OF SOME INGESTED INSECTICIDES ON THE MIDGUT WALL OF THE SOUTHERN ARMYWORM LARVA[1]

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INTRODUCTION

Although the specific action of insecticides on insects has received little attention in the past, the idea is gaining favor that in this field there are many problems that warrant special consideration. Further knowledge might prove useful in the selection, improvement, and application of methods for insect control. A review of the physical and chemical effects of poisons on insect tissues, cells, and secretions is given by Trappmann (*13*).[2] The histopathological effects on the midgut wall of the southern armyworm larva (*Prodenia eridania* (Cram.)) of certain poisons commonly used as insecticides have been studied by the writer in connection with an investigation of the normal anatomy of the alimentary canal, and are described in this paper.

MATERIALS AND METHODS

The poisons used were a high-grade acid lead arsenate, a fine fraction of paris green separated from a commercial lot, an average commercial grade of calcium arsenate, calcium arsenite,[3] arsenic trioxide, a finely divided commercial barium fluosilicate (20 percent silica), reagent-quality sodium fluoride, synthetic sodium fluoaluminate containing some uncombined aluminum oxide, chemically pure rotenone, and a high-grade commercial phenothiazine.

The larvae were reared on living turnip plants in a greenhouse insectary or on fresh turnip foliage in an incubator at 27° C. Actively feeding early sixth instars were removed from the plants and conserved for several hours on fresh, clean leaves in a clean glass cage, in order that any hard objects that might be present in the lumen of the gut would be eliminated. They were then held without food for several hours to induce hunger.

Lethal or unusually large doses of the poisons were fed to the larvae in turnip-leaf or sweetpotato-leaf sandwiches. The larvae were then held at 22°–27° C., and after different intervals were killed by immersion in hot water (60°–70°). The gut-wall tissue was prepared by the paraffin method for a microscopic study of the tissues and cells. Fixation was with Bouin's picro-formol-acetic. Sections 5 to 10 microns thick were cut in both the transverse and the longitudinal planes, stained with Ehrlich's haematoxylin, and counterstained with eosin or erythrosin. Similar preparations of larvae that had not ingested poisons were used for comparison.

[1] Received for publication April 13, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 328

[3] This product contained 45.7 percent As_2O_3, equivalent to 85 percent $Ca_3(AsO_3)_2$.

THE ALIMENTARY CANAL OF UNPOISONED LARVAE

The alimentary canal of the sixth-instar larva of the southern armyworm is similar to that of other noctuid larvae. The alimentary canal (pl. 1, *A*) is a straight tube extending from the mouth to the anus and has the usual three primary divisions. The foregut and the hindgut are each about one-third the length of the midgut. They are thin-walled and are lined with a sclerotic intima.

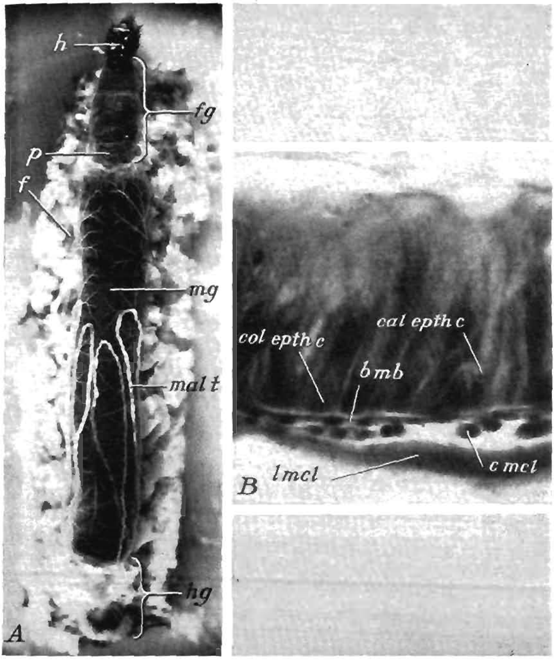
The midgut is a large, undifferentiated tube extending approximately throughout the first six abdominal segments. The canal is normally distended by ingested food materials and in this condition occupies most of the abdominal cavity. The midgut wall (pl. 1, *B*) is made up of an enveloping membrane known as the peritoneum, two layers of muscle fibers, those of the outer layer being longitudinal and those of the inner layer circular, a basement membrane, an epithelial layer, and a thin sheet of detached material closely surrounding the food mass and known as the peritrophic membrane.

The midgut epithelium (Pl. 2, *A*) consists of a single layer of different forms of epithelial cells, of which there are three principal types—columnar, calyciform, and interstitial. The columnar cells are variable in shape and appearance, depending in part on their location and physiological state. They are tall, and often cylindrical, but are usually enlarged distally with slender basal ends. The cell contains an elongate, coarsely granular nucleus. A striated border is present on the free surface of the cell. The slender basal ends of these cells rest on the basement membrane, and the surfaces of the enlarged distal ends are exposed to the gut lumen. The calyciform cells are shaped somewhat like a calyx and fill the spaces between the slender basal parts of the columnar cells, communicating with the free surface by a narrow neck. The cell is often expanded in its middle part. The nucleus lies at the basal end of the cell. Interstitial cells rest on the basement membrane between the bases of the other cells. They are round or elongate in section and contain a large nucleus surrounded by a small amount of strongly basophilic cytoplasm.

The epithelial cells probably function in the secretion of the juices required for digestion, and in the absorption of selected products of the digestion and their transference to the hemolymph. Absorption probably takes place largely, if not entirely, in the midgut.

HISTOPATHOLOGICAL PICTURE OF THE MIDGUT OF POISONED LARVAE

The usual observable effect of the poisons on the gut-wall tissues has been disintegration. The extent of damage has varied according to the kind of poison and the individual larva. The susceptibility of the insect's tissues to the action of the poisons and the quantity and distribution of the poison that reaches the affected areas are probably important factors in the individual variation. The extent of damage in individuals might be directly related to the initial dosage and the time over which the poison has acted, but these factors appear to be inseparable from individual variation unless large numbers of observations are made.



A, Alimentary canal of the sixth-instar southern armyworm exposed by incision along the middorsal line of the body wall, × 3; *B*, longitudinal section from midgut wall of a normal sixth instar, × 650. Abbreviations: *bmb*, basement membrane; *c mcl*, circular muscle fiber; *cal epth c*, calciform epithelial cell; *col epth c*, columnar epithelial cell; *f*, fat body; *fg*, foregut; *h*, head; *hg*, hind-gut; *l mcl*, longitudinal muscle fiber; *mal t*, Malpighian tubules; *mg*, midgut; *p*, proventriculus.

Longitudinal section from midgut wall: *A*, Showing normal epithelial cells, × 1200; *B*, showing injury to epithelial cells in larva poisoned by lead arsenate, × 1200.

ARSENICALS

The mode of action of arsenicals upon protoplasm has received considerable attention from the therapeutic standpoint (*1*, *14*, *15*), but not from the standpoint of insect control. The pathological effects of arsenic compounds on mammals have also been intensively studied, especially in connection with the problems of arsenic therapy (*2*, *6*, *10*). In the mammal a diversity of lesions is produced by different compounds of arsenic, and specificity of action is exhibited toward different species and toward different tissues. The injuries are usually definite structural changes in the cells, leading to disintegration.

Voskresenskaja (*16*) has made a chemical study of the degree and rate of penetration of arsenical compounds through the intestinal walls of several species of insects. Tareev and Nenjukov (*12*) report that sodium arsenite causes complete necrosis of the midgut of *Calliptamus*. Pilat (*11*) found that the gut epithelium of nymphs of *Locusta migratoria* L. had completely disappeared 96 hours after ingestion of this poison, and that the gut of caterpillars of *Aglais urticae* L. was severely damaged. There was no evidence of injury to the gut of larvae of *Pieris brassicae* L. 5½ hours after ingestion of sodium arsenite. Evidence has been presented (*7*, *9*) to show that sodium arsenite and other arsenicals may penetrate the integument of insects and become distributed in the underlying tissues and organs.

Observed effects on the southern armyworm midgut of some arsenicals commonly used in control of different species of insects are described below.

Lead Arsenate

By preliminary experiment it was determined that a dose of 0.5 mg. of the lead arsenate is lethal to sixth-instar larvae.

Doses of 0.5 to 1 mg. of the poison were fed to larvae, of which 30 were selected for study. The sandwiches containing the poison were readily consumed, generally within 20 to 30 minutes from the time they were offered. After ingesting the poison the larvae usually became inactive, refusing to feed even on fresh leaf. Within a few hours some of the larvae again became active, but did not feed. Larvae were killed and their gut walls fixed for study 2, 8, 9, 10, and 22 hours after the poison had been ingested.

Damage occurred in midgut walls of larva that had been active immediately before they were prepared for study, as well as of larvae that were already severely affected or apparently dead from the poison. The extent of damage was not clearly related to the time the larvae were held after ingestion of the poison. Apparently any part of the midgut wall may be affected.

The damage to the wall (pl. 2, *B*) is evident from the more or less complete disorganization of the cell structure. The various states of disintegration of epithelial cells are evidenced by the broken-down cytoplasm, vacuolization, merging of the cellular substance of adjacent cells, obliteration of the cell boundaries, disappearance of the striated border, and changes in the nuclei. Fragments of epithelial tissue are sometimes present in the lumen of the canal. Damage to the nuclei is shown in different ways. Part of the nuclear substance may be extruded beyond its original outline in bizarre forms. In some

individuals the nuclear material may be condensed; in others it is broken into particles of irregular form and size. Affected nuclei may stain lightly or not at all, their position being indicated by a faintly stained rim. A few nuclei contained vacuoles. The calyciform cells may be misshapen, or they may stand out distinctly and apparently with little or no damage to either the cell structure or the nucleus, although surrounded by columnar cells in advanced stages of disintegration. The peritrophic membrane is unchanged in appearance except in the most advanced stages of disintegration of the wall; it then may be absent in whole or in part.

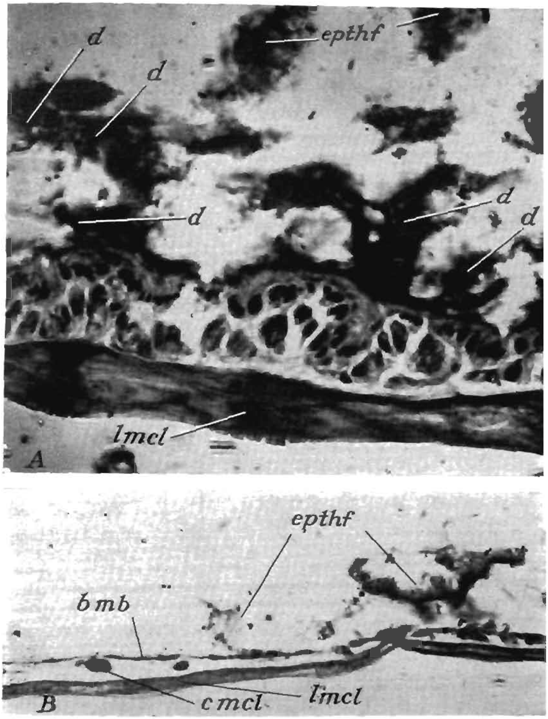
After the death of larvae from lead arsenate poisoning, the epithelium rapidly breaks down and sloughs off (pl. 3, *A*), leaving the basement membrane and muscle layers intact although the muscle fibers are obviously damaged (pls. 3, *B*, and 4, *B*).

A short time after death of the larvae, splotches of black material appear in the wall of the alimentary canal, particularly in the midgut. The material is distinctly visible in both unstained and stained preparations. It may be distributed over the wall as pin-point spots in such a way as to give the wall a sooty appearance. It also occurs in those parts of the Malpighian tubules, labial glands, fat body, and body wall that happen to be in contact with the affected areas of the alimentary canal wall (pl. 4, *A*). The occurrence of the deposit appears to be associated with the presence of the arsenic rather than the lead of the compound, since it did not appear after the larvae had ingested any of three other lead compounds—the acetate, chromate, or nitrate—but it did appear after they had ingested each of five other arsenicals—calcium, sodium, and potassium arsenates, and arsenic trioxide and pentoxide. The substance occurs as a deposit in the tissues of the midgut wall, and particularly in the muscle layers (pl. 4, *B*). The particles are found between the muscle fibers, but there appears to be no actual union with the tissue substance. Scattered deposits occur among the fragments of the disintegrating epithelial tissue (fig. 3, *A*).

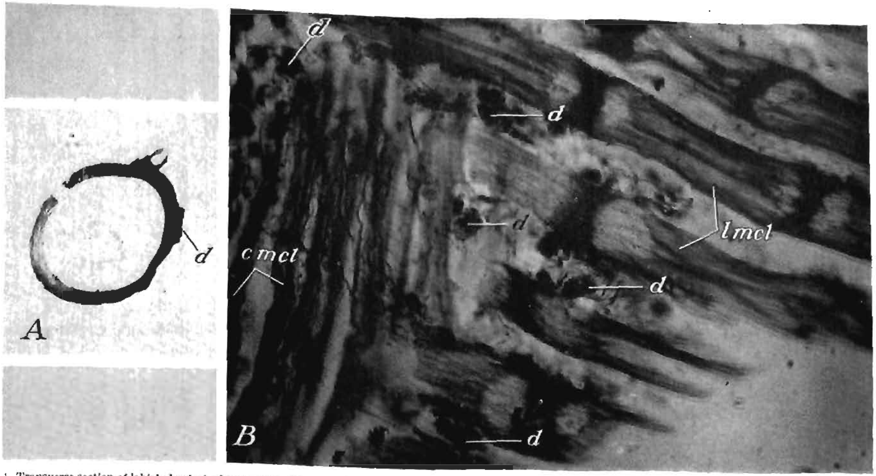
The deposition of arsenicals and other foreign substances in the tissues of animals has frequently been observed (3). The deposition and release of such stored substances are accomplished under more or less well-known conditions and may be followed by more or less well-known consequences. Arsenic may form insoluble compounds with proteins in the tissues of mammals, and by reaction with the hydrogen sulfide of the intestinal contents may produce insoluble sulfides. Consideration of the distribution of insecticidal substances in the organs and tissues of insects, and of the conditions affecting their possible storage and release, might lead to fruitful lines of investigation in insect toxicology.

Paris Green

A sandwich containing 0.5 mg. of paris green was fed to each of several larvae, of which six were selected for study. The sandwiches were completely eaten within 20 minutes from the time they were offered. Although supplied with fresh, unpoisoned leaves, the larvae fed no more after ingesting the poison. One larva was killed after 1 hour, at which time it was normally active. The other larvae were killed after 2½ to 3½ hours, at which time all showed only feeble signs of life.



Longitudinal section from midgut wall of larva poisoned by lead arsenate: *A*, Showing badly disintegratec and sloughing epithelium and presence of black deposit, × 700; *B*, showing almost complete disappear ance of epithelium, only the muscle fibers and basement membrane remaining, × 700. Abbreviations *bmb*, basement membrane; *c mcl*, circular muscle fiber; *d*, deposit of black material; *epthf*, epithelial frag ments; *l mcl*, longitudinal muscle fiber.



Transverse section of labial gland of a larva poisoned by arsenic trioxide, showing deposit of black material on side next to the alimentary canal wall in an area containing the black deposit, × 120; *B*, section taken in the plane of the muscle layer of the midgut of a larva poisoned by lead arsenate, showing the particles of black material between the muscle fibers, × 550. Abbreviations: *c mcl*, circular muscle fiber; *d*, deposit of black material; *l mcl*, longitudinal muscle fiber.

The histological sections showed the particles of paris green well distributed in the lumen among the food particles. The epithelial cells showed slight evidence of disintegration similar to that described for lead arsenate, but much less marked. The peritrophic membrane was apparently intact.

Pilat (*11*) found that the midgut epithelium of *Locusta migratoria* nymphs was very little damaged, if at all, 41 hours after ingestion of paris green, but that the epithelium of *Aglais urticae* caterpillars was injured.

Calcium Arsenate

Sandwiches containing 1 mg. of calcium arsenate each were offered several larvae, five of which ate the entire amount. These sandwiches were completely eaten within 1 hour after they were offered. Although supplied with fresh, unpoisoned leaves, the larvae fed no more after ingesting the poison, but regurgitated profusely. One larva was killed 1 hour after ingesting the poison, at which time it was active and apparently healthy. Two were killed 2½ hours after ingesting the poison, when they showed only feeble signs of life. The remaining two were killed 3 hours after ingesting the poison, at which time there was a marked shaking and twitching of the entire body. The tissues of the midgut wall were greatly disorganized, and the epithelial layer appeared as a rather solid mass, occasionally presenting fragments recognizable as cell structures.

Calcium Arsenite

Each of three larvae ate about one-third to one-half of a sandwich containing slightly more than 1 mg. of calcium arsenite. They were obviously very much affected within 2 hours, and after 3 hours they showed only feeble signs of life. The epithelium at this stage was highly disintegrated. The muscle layers were in place, but the fiber structure was broken up. According to Pilat (*11*), the midgut epithelium of *Locusta migratoria* nymphs and of *Aglais urticae* caterpillars is destroyed more or less, depending in part upon the interval between ingestion of the poison and dissection.

Arsenic Trioxide

Two larvae ingested approximately 1 mg. of arsenic trioxide each within half an hour. One larva was killed 2 and the other 3 hours after ingesting the poison, at which times they showed only feeble signs of life. The midgut epithelium of these larvae showed only slight, if any, injury due to the poison. A third larva ate an entire sandwich containing 5 mg. of the poison, ceased feeding immediately thereafter, and regurgitated profusely. It moved with difficulty after 5 hours. It was killed after 9 hours, at which time respiratory movements were the only signs of life. The entire midgut wall was severely damaged, as shown by merging of the cytoplasm and general disorganization of all the tissues of the epithelium.

O'Kane and Glover (*9*) demonstrated the passage of arsenic trioxide through the integument of *Periplaneta americana* (L.), and its distribution in the tissues of the internal organs.

BARIUM FLUOSILICATE

Sandwiches containing from 0.2 to 1 mg. of barium fluosilicate were readily ingested within about one-half hour. Eight larvae had eaten sandwiches containing 0.5 to 1 mg. of poison, and three others ate sandwiches containing 0.2 to 0.33 mg. After ingesting the poison one larva ate an unpoisoned sandwich and then became inactive. All others refused to eat, and all were inactive. Poison effects were shown by sluggishness, limpness, abnormal jerking movements with or without stimulation, and occasional regurgitation.

Larvae were killed and prepared for study 1, 3, and 5 hours after ingestion of the poison, and also when they appeared to be near death, which was usually within 8 or 9 hours.

In the histological preparations it was impossible to detect changes in the epithelium that could be attributed with certainty to the action of the barium fluosilicate. The individual cells showed little or no evidence of injury, and certainly no such disintegration as occurred with arsenicals. The peritrophic membrane was intact.

SODIUM FLUORIDE

Sodium fluoride in doses of 2 to 5 mg. was fed to the larvae. This quantity is lethal, death occurring within 3 to 12 hours. Two sandwiches containing 5 mg. of the poison were completely eaten within 1 hour, and several others were partially eaten within 2½ hours. After ingesting the poison, the larvae occasionally regurgitated and showed abnormal movements such as sudden rearing of the head and thorax twisting from side to side, and turning ventral side up. Affected larvae often were unable to crawl. They were inactive most of the time and refused to feed on fresh leaf. After a few hours they became limp.

Larvae were killed and their gut walls fixed for study 5 to 10 hours after ingesting the poison, at which time they usually showed only feeble signs of life, such as faint respiratory movements and slight activity of the appendages on stimulation with a needle.

Damage (pl. 5, *A*) to the midgut wall was shown in disintegration of the substance of the cytoplasm and nuclei of the epithelial cells. The cytoplasm of the columnar epithelial cells often appeared disrupted, usually at the distal or lumen end, giving this part of the cells a ragged appearance. Damage to the nuclei of the calyciform as well as to those of the columnar epithelial cells is shown in part in a fragmented and misshapen condition of the nucleus. Frequently the nuclei appear as solid-staining, apparently shrunken bodies, rather than as normal granular bodies. Occasionally the nuclei of the columnar cells had almost disappeared, being located merely by a stained ring representing the outline of the nucleus, and by scattered granules inside the ring. The calyciform cells present the usual outline and appearance of these cells. The peritrophic membrane was unchanged in appearance.

Pilat (*11*) found that the epithelium of *Locusta migratoria* nymphs and *Aglais urticae* caterpillars is damaged by sodium fluoride. Hockenyos (*5*) found that sodium fluoride can be absorbed in lethal amounts directly through the body integument of *Periplaneta americana* and *Blatta orientalis* L.

Longitudinal sections from midgut wall of poisoned larva: *A*, Larva poisoned by sodium fluoride, × 620; *B*, larva poisoned by sodium fluoaluminate, showing injury to epithelial cells, × 1400.

SODIUM FLUOALUMINATE

Five larvae each readily ingested a sandwich containing 1 mg. of ynthetic sodium fluoaluminate within 30 minutes. They were then uiescent for about an hour, after which they again became active without immediately showing symptoms of poisoning.

Two larvae were killed and their gut walls prepared for study 9 hours fter they had ingested the poison, at which time one was unusually luggish and the other jerked its body in an abnormal manner. Three arvae were killed 24 hours after ingesting the poison, at which time hey were active and showed no such abnormal behavior.

The midgut walls of all these larvae were greatly disintegrated, and here was no clear distinction in effects between the larvae killed hours and those killed 24 hours after ingesting the poison. The cross triations of the muscle fibers were faint or obliterated. Damage to he epithelium (pl. 5, *B*) was seen from the disorganization of the pithelial cells, merging of the cytoplasm of adjacent cells, and changes n the nuclei. The most frequently observed evidence of damage to he nuclei was fragmentation. In the same microscopic field with ragmented nuclei would often be others more or less shrunken and eeply stained, and still others apparently in the process of fading out. Exfoliation of the entire epithelial layer from the subjacent basement membrane occurred over extensive areas.

PHENOTHIAZINE

One larva was offered a sweetpotato-leaf sandwich containing pproximately 1 mg. of phenothiazine. It immediately ate small ortions from the edge of the sandwich, and then fed no more either n the sandwich or on fresh leaf. Within 24 hours it had become labby and apparently lifeless except for feeble movements when ouched with a needle. There was no obvious change in its condition fter 60 hours. At this time a lighted electric bulb was brought near he larva, causing movements of the thoracic legs, followed by a more igorous movement in which the fore part of the body was thrown ackwards until it touched the hindermost part of the body. When he bulb was removed, the larva immediately resumed its apparently ifeless and flabby appearance.

The larva was then killed. Microscopical examination revealed no tructural differences in any of the tissue elements of the midgut that ould with certainty be attributed to an action of the phenothiazine. Even the delicate striated border was remarkably well preserved.

ROTENONE

Five southern armyworm larvae each ingested a sweetpotato-leaf andwich containing 5 mg. of fine, chemically pure rotenone. The arvae were then placed on a living turnip plant, upon which they fed nd reacted normally. From 75 to 84 hours later there were no changes n the microscopic appearance of the epithelial cells or other tissues of he midgut that could with certainty be attributed to rotenone.

Rotenone dissolved in olive oil may cause severe hyperemia of the ntire digestive tract of white rats (*8*). The effects of rotenone upon ish (*4*) consist primarily in the destruction of the gill epithelium, which makes the inspiration of oxygen impossible.

Rotenone varies remarkably in its toxic action on different species of insects. Ingested rotenone apparently has no toxic action on the southern armyworm, and most of it passes through the insect unchanged (*18*). Cube, in which rotenone is the principal insecticidal ingredient, also is apparently ineffective as a stomach poison against the southern armyworm (*17, pp. 62–63*).

In the present work silkworm larvae died within about 2 hours from the effects of ingesting minute quantities of rotenone, whereas southern armyworm larvae readily ingested 5 to 10 mg. of rotenone in sandwiches without showing any ill effects.

SUMMARY

Histopathological effects of certain insecticides on the midgut wall of the sixth-instar southern armyworm (*Prodenia eridania* (Cram.)) were studied. Lethal or large doses of poisons were fed by the sandwich method, and after sufficiently long intervals of time the larvae were killed and the tissues prepared by histological methods. Similar preparations of larvae that had not ingested poisons were used for comparison.

The different insecticides showed differences in action ranging from no apparent effects to well-marked damage to the midgut wall. The ingestion of arsenicals (lead arsenate, paris green, calcium arsenate, calcium arsenite, and arsenic trioxide) was followed by disintegration of the midgut epithelial cells and damage to the midgut muscle fibers. Disintegration of the substance of the cytoplasm and nuclei of the epithelial cells followed the ingestion of sodium fluoride. The epithelial cells of larvae that had ingested sodium fluoaluminate were greatly disintegrated, and the cross striations of the muscle fibers were faint or obliterated. The ingestion of barium fluosilicate, phenothiazine, and rotenone was followed by no changes in the epithelium or muscle fibers that could be attributed with certainty to these substances.

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THE INFECTION CAPABILITIES OF HOP DOWNY MILDEW[1]

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INTRODUCTION

Downy mildew, *Pseudoperonospora humuli* (Miyabe and Tak.) Wils, was first observed in commercial hopyards in Oregon in 1930. Following a survey to determine the incidence of infection, an investigation of the life history and infection capabilities of the causal organism was inaugurated. The primary objectives of the investigation were to determine the host range of the fungus; to discover any evidence of resistance to the fungus among botanical species, horticultural varieties, or strains of hops developed by means of selection or hybridization; and by attempting to infect all available known hosts of other species of the genus *Pseudoperonospora*, to confirm or disprove the opinion [2] that physiological races of the fungus may exist.

MATERIALS AND METHODS

In a previous paper the writer [3] discussed in some detail materials and methods employed and results obtained early in the course of this investigation.

In addition to hop seedlings, the leaves of hop plants grown from cuttings were employed. Both cotyledons and leaves of seedlings, as well as the leaves of more mature plants of other host genera, were used. In some instances excised cotyledons and leaves were incubated after inoculation in Petri dish moist chambers or floated in covered Petri dishes of nutrient or sugar solutions.

The methods of applying the inoculum varied also. In addition to the use of camel's-hair brushes for the transfer of inoculum, water suspensions of zoosporangia were atomized onto the plant parts or placed in localized areas by means of a medicine dropper.

HOST RANGE

An extensive series of inoculations with the zoosporangia of the hop downy mildew fungus was begun in 1931 and continued each year through 1937.

The original inoculum was obtained from naturally infected hop plants in Oregon yards.

Only positive results are recorded here. Optimum environmental conditions were not subject to accurate control, and to this circum-

[1] Received for publication June 17, 1940. Cooperative investigation by the Division of Drug and Related Plants, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station. Technical Paper No. 295, Oregon Agricultural Experiment Station.

[2] SALMON, E. S., and WARE, W. M. THE DOWNY MILDEW OF THE HOP IN 1930. Inst. Brewing Jour. 37 (n. s. 28): 24–32, illus. 1931.

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stance some of the negative results obtained doubtless can, in par at least, be attributed.

Results are herein recorded as positive when the organism invadin the infected host developed zoosporangia, whether or not accompanie by distinctly visible lesions on the inoculated parts not found o similar uninoculated parts.

Source of inoculum	*Host infected*
Humulus lupulus L.	
Variety unknown	*Humulus lupulus* L.:
	Early Clusters.
	Early Green.
	East Kent Golding.
	Fuggles.
	Late Clusters.
Early Clusters	*Celtis mississippiensis* Bosc.
	C. occidentalis L.
	C. sinensis Pers.
	C. tournefortii Lam.
	Humulus lupulus:
	Early Clusters.
	Fuggles.
	Late Clusters.
	Red Vine.
Fuggles	*Humulus lupulus:*
	Early Clusters.
	Fuggles.
	Late Clusters.
	Red Vine.
Late Clusters	*Cannabis sativa* L.:
	Bologna.
	Kymington.
	Palmate.
	Pinnatifid Simple Leaf.
	Simple Leaf.
	Tochigi.
	Celtis mississippiensis.
	C. occidentalis.
	C. sinensis.
	C. tournefortii.
	Humulus japonicus Sieb. and Zucc.
	H. japonicus var. *variegatus* Hort.
	H. lupulus:
	"Bohemian."
	Early Clusters.
	East Kent Golding.
	Fuggles.
	Late Clusters.
	Red Vine.
	H. lupulus var. *neo-mexicanus* Nels. and Ckll.
	Urtica lyallii Wats.
Red Vine	*Humulus lupulus:*
	Fuggles.
	Red Vine.
Humulus lupulus L. var. *neo-mexicanus* Nels. and Ckll.	*Humulus lupulus:*
	Fuggles.
	Late Clusters.
	Red Vine.
	Humulus lupulus var. *neo-mexicanus.*
	Urtica lyallii.
Urtica lyallii Wats.	*Humulus lupulus:*
	Fuggles.
	Late Clusters.
	Urtica lyallii.

It is of interest, in passing, to note that all attempts to infect ailable hosts of *Pseudoperonospora cubensis* (B. and C.) Rostowzew, *erodii* (Fckl.) Wils., and *P. portoricensis* (Lamkey) Hoerner[4] were nsuccessful. Host material of *P. elatostemae* (Togashi and Onuma) loerner[4] was not available.

RESISTANCE

Of the botanical species of hops inoculated, *Humulus japonicus* nd *H. japonicus* var. *variegatus* showed some evidence of resistance. 'his fact is of doubtful economic importance, however, since both pecies are annuals and may not, in view of present knowledge,[5] be uitable for hybridization with the perennial species and varieties f *H. lupulus*, from which many of the hops of commerce are derived.

In addition to the inoculation work recorded above, field observaons were made and additional inoculation work was carried on with number of standard varieties, seedlings, and strains of hops develped by selection or hybridization at the Oregon Agricultural Experiient Station in cooperation with the Bureau of Plant Industry, in earch for possible immunity to downy mildew. No immunity was ound. Apparent differences in susceptibility which have appeared in ertain lines have not maintained themselves consistently in subseuent tests. The evidence leads to the conclusion that individual lants may escape infection, for various reasons, for several years, lthough they are not genetically resistant.

The development of zoosporangia resulting from inoculation was elatively sparse on all other hosts as compared with that on *Humulus* pp. Large hypersensitive areas appeared consistently on the cotyedons of *Celtis occidentalis* and on both cotyledons and leaves of *. mississippiensis, C. sinensis,* and *C. tournefortii.* Similar evidence "subinfection" was a common occurrence also on the leaves of *'rtica lyallii.*

The hypersensitive areas referred to were definitely necrotic but definite in outline (fig. 1). The surface, in time, usually became epressed in relation to the surrounding normal tissue. Affected ssue became discolored through varying shades of yellow, brown, or ack. Oftentimes no zoosporangia were produced in the necrotic ea; when produced, they were noticeably fewer than those produced infected plant parts not developing the hypersensitive areas.

These phenomena indicate that the different host species provided fferent physiological environments for the invading organism.

In view of the fact that all species tested of the genera *Cannabis, eltis, Humulus,* and *Urtica,* which are hosts respectively of *Pseudoeronospora cannabina* (Otth) Hoerner, *P. celtidis* (Waite) Wils., *. humuli* (Miyabe and Tak.) Wils., and *P. urticae* (Lib. ex Berk.) alm. and Ware, became infected with *P. humuli,* it would seem not utside the range of possibility that they may, after all, prove to be ly different physiological races of one of the species of the genus *seudoperonospora.*

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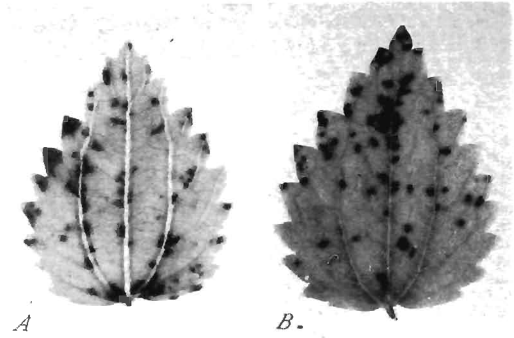

FIGURE 1.—Lower surface (*A*) and upper surface (*B*) of nettle leaf (*Urtica lyalli* infected with hop downy mildew (*Pseudoperonospora humuli*), showing hype sensitive areas.

SUMMARY

The host range of the hop downy mildew fungus was found to l considerably more extensive than had previously been reported. A hosts, however, were members of the nettle family.

Of the botanical species of hops inoculated, *Humulus japonicus* an *H. japonicus* var. *variegatus* showed some evidence of resistance Evidence of resistance on the part of horticultural varieties or strair of hops developed by means of selection or hybridization was incor clusive. Further study to confirm preliminary indications is necessar before definite conclusions would be justified.

In view of the infection by the hop downy mildew of species c *Cannabis*, *Celtis*, and *Urtica*, hosts respectively of *Pseudoperonospor cannabina*, *P. celtidis*, and *P. urticae*, it is suggested that the form of *Pseudoperonospora* described under these names, along with *I humuli*, may eventually prove to be physiological races of a singl species.

CHEMICAL AND PHYSICAL PROPERTIES OF SOILS AND OF THEIR COLLOIDS DEVELOPED FROM GRANITIC MATERIALS IN THE MOJAVE DESERT[1]

By I. C. Brown, *associate chemist, Division of Soil Chemistry and Physics*, and M. Drosdoff, *assistant chemist, Division of Soil Survey, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The opinion has been prevalent that desert soils for the most part are composed of sandy and gravelly materials which are loose and porous in character except for local areas of lime and gypsum hardpans. Lapham (*22*)[2] objected to this view and suggested that this concept developed through theoretical considerations and from the fact that the early studies of Hilgard and others were largely directed to areas of slightly weathered alluvial soils which were well situated with reference to water supply for irrigation. Thus Hilgard (*16*) stated that in the arid regions "the formation of colloidal clay is very much diminished, so that most soils formed under arid conditions are of a sandy or pulverulent type."

More recent studies of soils in arid regions indicate that the formation of soils with considerable clay content is common in areas of limited rainfall, both in the northern and southern deserts. In many of the recent soil surveys of southern Arizona and California, typical mature desert soils are described in which the subsoil contains a relatively large amount of clay (*21, 31*).

Nikiforoff (*26*), in a morphological study of the soils of the Mojave Desert, found that one of the principal zonal characteristics of southern desert soils is the presence of a reddish-brown clay layer, or "claypan," in the lower part of the solum. In areas of higher rainfall the cause of claypan formation is assigned by Brown, Rice, and Byers (*6*) to the flocculation and filtering out of the eluviated colloid. Bray (*3*) concluded that in certain Illinois soils a claypan is formed by vertical transfer of clay without much alteration in its composition. Nikiforoff (*26*) did not find any evidence in the field study of the Mojave Desert soils to indicate an illuvial origin of the claypan. He suggested that the clay layer is formed in place by the hydrolytic decomposition of the primary minerals. It seemed desirable to make a laboratory study of the soils of this area in order to obtain further evidence bearing on this hypothesis and to add to our meager knowledge of the chemical composition of desert soils.

DESCRIPTION OF THE SOILS

The soils were collected by C. C. Nikiforoff, of the Division of Soil Survey, and the junior author. Special care was taken to select soils with a more or less distinct claypan; one soil, however, without such a horizon (profile F) was also sampled as a representative of the

[1] Received for publication August 3, 1940.
[2] Italic numbers in parentheses refer to Literature Cited, p. 350.

common but apparently underdeveloped soils of the alluvial fans the desert.

The samples were collected in the southwestern part of the Moja Desert. Five profiles were selected within a few miles of Mojave, Kern County, and one profile (B) in San Bernardino County, both California.

The climate of the Mojave Desert is arid and hot. It is of t so-called Arizona type that is characterized by a distinct winter co centration of rainfall. The mean annual rainfall is slightly less th 5 inches, and more than three-fourths of it falls during the wint The mean annual temperature is about 65° F.

The winter rains are gentle and practically all the rainfall is absorb by the soil, which usually becomes moist during this period to a dep ranging from about 2 to more than 5 feet, depending upon the annu variation of the seasonal rains. This moisture permits the seeds annual plants to germinate and supports the short but sometimes ve spectacular spring outburst of desert flora. The return of the usu summer aridity and heat of the desert quickly terminates the veget tive period. The rains during the summer are rare and usually fall heavy showers of short duration. Most of this water is lost throug run-off; it seldom penetrates the soil more than 1 inch, and the sc dries up almost as fast as the rain passes. Only the most drough resistant perennial plants survive throughout the year; the most typ cal of these are the creosotebush (*Larrea divaricata* Cav.), certa sages, the Joshua-tree (*Yucca brevifolia* Englem.), and sever varieties of cactus.

As the area in which the soil samples used in this investigation we collected has not been covered by a detailed survey, the soils have no as yet been correlated. The soils developed from a residual grani material (profiles A, B, and C) are tentatively grouped into the Muro series. This name, however, is subject to possible change when th final correlation is made. The soil represented by profile E is ver similar to the soils of the Mojave series. No names were suggeste for the soils represented by profiles D and F.

A short description of each profile as furnished by the collecto follows:

PROFILE A

Location.—Mojave desert, 7.75 miles east of Rosamond, along the road to Muroc and about 2.25 miles north of highway. Elevation about 2,900 feet. A gentle slope to the south from the base of a low rocky ridge. Surface drainage is good.

Vegetation.—Widely spaced creosotebushes and other desert shrubs; a few single Joshua-trees. Much of the surface between the shrubs is bare and covered with a thin coat of angular grit.

Parent material—Very gritty and derived from granite. Rotten rock generally occurs at a depth ranging from a few inches to more than 2 feet from the surface. Numerous outcrops of bedrock are scattered all over the area.

Description of the profile:

1 (?). 0–2 inches. Probably overwash. Loose gritty loam of a medium brownish-gray color.

2 (B_1). 2–5 inches. A very compact gritty clay of a bright dark reddish-brown color; sticky when wet, hard and massive when dry.

3 (B_2). 5–11 inches. Practically the same texture, color, and consistence as the overlying horizon but differs from the latter in structure. It breaks into well-defined roughly prismatic clods. The surfaces of these clods are much darker than the interior.

4 (B_3). 11–14 inches. Gritty clay of a less brilliant and somewhat lighter reddish-brown color. It breaks on drying into coarse irregular lumps.

5 (C). 14–34 inches. Rotten granitic rock. To about 40 inches from the surface this material has a pinkish-brown to rusty-brown color. Further down the pink and brown tints disappear and the disintegrated rock retains the light-gray color of the original solid granite.

The free carbonates appear at the depth of 12 inches from the surface. In B_3 subhorizon, a weak pseudomycelium is formed. In the disintegrated rock, carbonates form numerous anastomosing mostly horizontal streaks, ranging in thickness from a mere film to more than 1 inch.

PROFILE B

Location.—Mojave desert, 7.4 miles north of Kramer corner (crossroad 35.5 miles east of Mojave). About 300 feet west of highway. Elevation about 3,000 feet. Low, very gently sloping granitic ridge. Surface drainage is good.

Vegetation.—Sparsely scattered desert shrubs with a practically bare surface between them.

Parent material.—Residual from granitic rock.

Description of the profile:

1 (?). 0–7 inches. Probably overwash. Friable gritty loam of a dull medium- to light-brown color. Its thickness at different places ranges from less than 5 inches to about 2 feet.

2 (B_1). 7–10 inches. Compact gritty clay loam of a bright but rather dark reddish-brown color. It breaks on drying into roughly prismatic clods.

3 (B_2). 10–14 inches. The same color, texture, and consistence as B_1, but it does not crack as much. It is highly calcareous and differs in this respect from B_1, which is practically free from carbonates. The carbonates form numerous pure-white segregations, ranging in diameter from a small fraction to about 1 inch.

4 (B_3). 14–24 inches. Strongly weathered gritty granitic material impregnated with abundant streaks formed by carbonates.

PROFILE C

Location.—10.9 miles east of Mojave along the highway to Barstow and 1 mile north of the highway. Elevation about 2,800 feet. Undulating to gently rolling topography of a wide and low granitic ridge. Surface drainage is good.

Vegetation.—Sparse creosotebushes and other desert shrubs. Practically bare surface between the bushes.

Parent material.—Residual from granite. Rotten rock occurs at a depth ranging from less than 1 to more than 2 feet.

Description of the profile:

1 (?). 0–6 inches. Loose, structureless, gritty sandy loam of a light grayish-brown color.

2 (A_2). 6–10 inches. Somewhat heavier in texture, slightly more reddish in color and considerably more compact than 1.

3 (B). 10–17 inches. Gritty clay loam of a medium-brown to reddish-brown color and of a compact but crumbly consistence. Breaks on drying into poorly defined but roughly prismatic clods.

4 (C_1). 17–26 inches. Crumbly, disintegrated granite of yellowish- to rusty-brown color, with numerous red spots and films in cracks.

5 (C_2). 36–48 inches. Light-gray disintegrated granite, considerably less crumbly than C_1.

Carbonates appear at a depth of about 9 or 10 inches from the surface.

PROFILE D

Location.—3.55 miles north of Mojave along the highway to Bishop. Elevation about 3,000 feet. Broad alluvial fan, very gently sloping eastward from the base of Tehachapi Mountains toward the playa.

Vegetation.—Creosotebushes, with a few scattered Joshua-trees. Practically bare surface between the shrubs.

Parent material.—A gritty indistinctly stratified water-laid deposit.

Description of the profile:

1 (?). 0–12 inches. Probably mechanical overwash rather than a genetic horizon. Structureless gritty loamy sand to gritty sand of a light-brown color and of a friable consistence. It is covered by a thin somewhat compacted but fragile crust underlain by a porous (vesicular) material of very light-gray color extending from 1 to 2 inches from the surface.

2 (B). 12–24 inches. Gritty sandy clay loam of a bright-brown or reddish-brown color and of a compact consistence. It is sticky when wet and breaks into irregular, roughly prismatic clods when dry.

3 (C_1). 24–36 inches. Brittle, weakly carbonate cemented hardpan, composed of gritty sandy clay loam with a small admixture of gravel.

4 (C_2). 36+ inches. Crumbly to friable gritty sand of light grayish-brown color. Content of gravel increases gradually in deeper strata, but stratification is rather indistinct. Segregation of carbonates in pockets and streaks produces a slight local cementation.

Carbonates appear at a depth of about 27 inches from the surface. At a depth of 50 inches and deeper, the content of carbonates increases and they segregate in crumbly, more or less horizontal streaks ranging in thickness from a mere film to more than 1 inch.

PROFILE E

Location.—3 miles east of Mojave along the highway to Barstow. Elevation about 2,700 feet. Broad flat at the base of Tehachapi Mountains. The general surface is nearly level, marked by a slightly developed microrelief. Surface drainage is fair.

Vegetation.—Sagebrush and other desert shrubs, with very scant growth of annuals between the shrubs. Areas of practically bare surface are numerous.

Parent material.—Presumably stratified old water-laid deposits (basin filling) of undetermined thickness.

Description of the profile:

1 (?). 0–5 inches. Probably a recent mechanical overwash, loose, structureless, gritty loam of a medium grayish-brown color.

2 (B_1). 5–10 inches. Gritty clay loam of a reddish-brown color and very compact consistence. Breaks into coarse angular clods when dry; massive and very sticky when wet.

3 (B_2). 10–20 inches. Gritty clay loam of a much brighter and somewhat darker reddish-brown color and of a somewhat heavier texture than in the B_1. It has a very compact consistence and breaks into well-defined, roughly prismatic clods on drying.

4 (B_3). 20–34 inches. Gritty clay loam of a somewhat lighter and duller color than that of B_1 and B_2; more or less massive, very compact, and moderately cemented by carbonates. The carbonates form a very fine moldlike pseudomycelium that thoroughly penetrates the subhorizon. A small admixture of gravel is present.

5 (C). 34+ inches. Loose coarse sand, grit, and gravel mixed indiscriminately. General color is light grayish brown.

PROFILE F

Location.—4 miles north of Mojave along the road to Bishop and 1 mile east of the highway. Elevation about 2,800 feet. Lower part of a broad alluvial fan. An exceedingly gentle general slope eastward. Surface drainage is good.

Vegetation.—Widely spaced growth of creosotebushes; an occasional Joshua-tree. Between the creosotebushes are interspread *Grayia spinosa* (Hook.) Moq., *Cassia armata* Wats., *Franseria dumosa* Gray, and several other desert shrubs, *Eriogonum inflatum* Torr., *Orytheca perfoliata* Torr. and Gray, *Gilia*, etc.

Parent material.—An indistinctly stratified fan alluvium several hundred feet thick.

Description of the profile:

1 0–3 inches. Very porous (vesicular) and friable sand of very light-gray color. Its surface is compacted into a thin crumbly crust covered by a coat of granitic grit.

2 3–16 inches. A structureless friable sand to loamy sand with a small admixture of granitic grit; very light brown in color.

3 16–48 inches. Similar to the above horizon except for a somewhat finer texture near the base and a moderate content of carbonates, which are disseminated throughout the material without any distinct efflorescence.

4 48+ inches. A structureless sandy loam of a dull-brown color thoroughly mottled with a rich pseudomycelium of carbonates. It is rather mellow in a moist condition but bakes on drying into a massive and slightly cemented pan.

METHODS OF EXAMINATION

The methods used in determining the various constituents and properties of the samples of soils and the colloids extracted from them were essentially those in general use by the Division of Soil Chemistry and Physics. They have been described in detail in the publications cited below.

All of the soils were air-dried before making the laboratory determinations. The mechanical analyses were made by the pipette method described by Olmstead, Alexander, and Middleton (*27*), except for the added changes mentioned by Knight (*20*). As this method requires the removal of organic matter, the approximate percentages obtainable by this procedure are also recorded. The hydrogen electrode described by Bailey (*2*) was used in determining the pH value of the soil. The soluble salts were determined by the bridge method described by Davis (*8*). The ratio of water to soil was 10 to 1.

The chemical analyses of both the soil and colloid were made according to the procedure described by Hillebrand (*17*) and as outlined by Robinson (*29*). Replaceable bases and base-exchange capacity were determined at pH 7.0, normal ammonium acetate being used as the replacing agent.

The procedure used for extracting the colloids from the soils has been described by Brown and Byers (*4*). No dispersion agent was used. The soil was dispersed in 3 gallons of distilled water, by means of a mechanical stirrer described by Holmes and Edgington (*18*). The suspension was decanted into 7 gallons of water and centrifuged at the rate of 17 seconds per liter at a speed of 16,500 revolutions per minute (bowl diameter, 4 inches). The colloid, still in suspension, was collected on a Pasteur-Chamberland filter, and the filtrate was used again to disperse the sediment removed from the centrifuge bowl. The process was repeated two to four times, until an adequate amount

of colloid was obtained. Very few of the colloid particles exceed 0.3μ in diameter; most of them were much smaller.

The data are presented in separate tables. Certain derived da have been segregated. In these, only the oxides of magnesiu calcium, potassium, and sodium are referred to as bases. In calc lating certain derived data, the percentage of each constituent divided by its formula weight. These quotients are compared in t ratios.

ANALYTICAL RESULTS

The mechanical composition of the six profiles is presented in tab 1. The analyses in this and in other tables are divided into tw parts: (1) Soils derived from granite and (2) soils derived from alluvi fan materials. Each of these divisions is arranged in the order of t profile development of the claypan in them. In these mechanic analyses the usual procedure of using only the portion consisting particles less than 2 mm. in diameter is adopted, as this is the portio usually defined as soil. With these and other data, some of the po sible causes of claypan formation in desert soils will be explore

TABLE 1.—*Mechanical analyses of Mojave Desert soils* [1]

DERIVED FROM GRANITE

Profile and sample No.	Horizon	Depth	Fine gravel (2-1 mm.)	Coarse sand (1-0.5 mm.)	Medium sand (0.5-0.25 mm.)	Fine sand (0.25-0.1 mm.)	Very fine sand (0.1-0.05 mm.)	Silt (0.05-0.002 mm.)	Clay (0.002-0 mm.)	Organic matter from H_2O_2	Less than 0.005 mm.
Profile A:		*Inches*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percen*
C3419	1	0-2	21.6	16.8	6.2	10.2	10.7	24.8	9.0	0.6	13.
C3420	2	2-5	16.7	15.1	5.3	9.0	7.3	10.2	35.9	.4	38.
C3421	3	5-11	19.0	12.0	3.7	7.0	9.7	13.6	34.5	.3	36.
C3422	4	11-14	11.7	9.9	4.3	9.8	14.7	19.6	29.5	.3	32.
C3423	(2)	14-34							14.0		
C3424	(2)	44-50									
Profile B:											
C3410	1	0-7	16.2	22.4	13.2	16.9	6.8	9.5	14.6	.2	17.
C3411	2	7-10	14.3	19.2	11.4	15.1	6.0	9.6	24.0	.2	26.
C3412	3	10-14	15.8	18.9	9.6	12.6	5.1	10.3	27.3	.2	29.
C3413	4	14-24	41.4	22.2	6.0	5.7	2.0	7.2	15.0	.4	17.
Profile C:											
C3401	1	0-6	15.4	14.9	9.1	21.2	11.5	14.0	13.7	.1	16.
C3402	2	6-10	9.7	10.6	8.5	24.9	14.8	15.3	16.1	.0	18.
C3403	3	10-17	19.4	13.7	7.6	16.2	8.9	13.6	20.3	.1	22.
C3404	4	17-26	53.6	22.4	4.3	4.2	1.5	6.7	7.2	.0	9.

DERIVED FROM ALLUVIAL FAN MATERIALS

Profile and sample No.	Horizon	Depth	Fine gravel (2-1 mm.)	Coarse sand (1-0.5 mm.)	Medium sand (0.5-0.25 mm.)	Fine sand (0.25-0.1 mm.)	Very fine sand (0.1-0.05 mm.)	Silt (0.05-0.002 mm.)	Clay (0.002-0 mm.)	Organic matter from H_2O_2	Less than 0.005 mm.
Profile D:											
C3395	1	0-3	8.7	22.2	14.3	23.7	10.9	10.9	8.6	0.4	12.
C3396	2	3-12	9.2	23.8	16.5	27.7	9.0	4.2	9.5	.0	10.
C3397	3	12-24	8.2	15.6	11.3	20.0	10.7	10.3	23.8	.0	26.
C3398	4	24-30	11.0	16.6	11.6	22.0	8.8	9.2	20.7	.0	22.
C3399	5	30-36	25.4	19.4	10.5	16.7	6.2	7.6	13.9	.1	16.
C3400	6	36-50	32.5	28.7	12.9	11.4	2.1	3.4	8.3	.6	8.
Profile E:											
C3414	1	0-5	12.0	16.8	12.1	24.1	12.1	8.8	13.6	.3	16.
C3415	2	5-10	7.3	12.2	10.1	23.9	14.8	9.1	22.5	.0	25.
C3416	3	10-20	12.0	17.3	10.8	17.7	7.7	7.2	26.7	.4	28.
C3417	4	20-34	10.7	19.9	14.2	21.3	5.6	6.5	21.2	.3	23.
C3418	5	34-44	22.2	25.5	15.0	18.3	4.3	4.7	9.9	.0	10.
Profile F:											
C3879	1	0-3	13.0	25.2	16.8	24.8	9.5	6.2	4.1	.2	4.
C3880	2	3-6	6.8	19.2	16.3	30.9	10.6	9.3	6.7	.0	7.
C3881	3	6-16	6.3	19.0	16.1	31.2	12.1	0.7	8.5	.0	9.
C3882	4	16-24	12.5	24.3	15.6	26.1	8.7	5.0	7.7	.0	8.
C3883	5	24-40	5.4	21.0	17.7	34.5	10.8	3.6	6.9	.0	7.
C3884	6	40-48	2.3	13.5	16.2	16.9	35.9	6.2	8.9	.0	9.
C3885	7	48-60	2.3	5.4	7.2	25.8	19.0	25.6	14.5	.0	20.

[1] Analyses by T. M. Shaw, E. F. Miles, and A. E. Yelmgren.
[2] Broken rock.

The first five of the profiles have a marked accumulation of clay in he part commonly defined as the B horizon. The last profile does not. The surface horizon of each profile may be overwash, as indicated in the descriptions of the profiles.

In profile A, horizon 2 has nearly 36 percent clay, and horizon 3 has only a little less. Horizon 4 rests on the broken parent rock and contains still less clay. Profiles B and C are similarly developed, though the percentage of clay in the subsoil horizons is not so high as in A.

The last three profiles in table 1 are developed on alluvial fan materials. The character of these profiles is necessarily somewhat different from that of the residual soils. It is evident that their texture may be modified by the quantity, the kind, and the size of rock fragments poured out and deposited on the alluvial fans by the ntermittent torrential floods issuing from the often dry beds of mountain streams. The upper, more recently deposited layer, of course, would be less modified by profile development than the deeper parts. The character and age of the whole deposit, for that matter, would greatly influence the character of the soil. Most of the material in each profile appears to be derived from granite. Profiles D and E have distinct clay layers in the subsoil horizons. Although from the field evidence there is some question as to whether the clay was formed as the soil developed or whether it was water-deposited, here are indications that the clay has been developed in place as a esult of soil-forming processes. Profile F has no clay layer and ppears to be composed of more recently deposited stratified layers of oarse material.

In an attempt to discover the source of the formation of so large a proportion of clay in certain horizons, a preliminary mineralogical examination of the whole soil and of the sand fractions of a few profiles was made. This examination showed, as Brown and Drosdoff (*5*) have reported, that alteration of the feldspar is marked and he less easily decomposable minerals have also been altered. It was evident, however, that, although losses have occurred only minor differences in the mineralogy of the profiles are distinguishable.

While the mechanical analysis was being made, it was found that he coarse particles of the partly weathered biotite mica exfoliated eadily when treated with hydrogen peroxide (*11*). This was caused by the presence, between the mica plates, of manganese dioxide, which decomposed the peroxide and caused exfoliation. This resulted in an increase in the coarse separates and a consequent decrease n the finer fractions, owing to the cohesion of the exfoliated plates of mica when the soil was heated after the peroxide treatment. However, the differences were not significant because of the relatively mall percentage of this hydrated biotite mica.

The results of chemical analyses of the soils (table 2) indicate their granitic origin, although the soils of profiles D, E, and F are derived rom transported alluvial fan material. The silica seldom exceeded 70 percent and was never less than 60. The sesquioxides and the bases fall well within the percentages occurring in granites. All parts of the profiles have been altered by weathering. Hydrolysis of the minerals has taken place, and the soluble material has been removed rom the residue by the small amount of water that percolates through

the soil during the rainy season, as indicated by the small amount of soluble salts present. The soils are, nevertheless, alkaline throughout. The pH value was never less than 7.4 nor greater than 9, and was usually about 8. The lowest alkalinity usually occurred in the claypan horizons, where weathering tended to be greater than in other parts and the soluble bases had been removed.

The chemical analyses of the soils of the alluvial fans do not always show the effect of degradation of the primary minerals in the more continually moist claypan zone. The heterogeneous material of the alluvial fan is, however, largely derived from granitic sources, but the irregularities of deposition, in amount, time, and place, produce a condition that is only imperfectly overcome in the formation of a profile.

The results of chemical analyses of the colloids are presented in table 3. Decomposition of the primary minerals of the soil, pronounced losses of silica and sodium by leaching, and consequent increases of the sesquioxides, magnesium, and the ignition loss of the colloids, as compared with the analyses of the soils in table 2, are immediately apparent. Contrary to expectation, the low rainfall of the desert has supplied sufficient moisture for hydrolysis of the parent minerals and an effective seasonal leaching of the products in times of great supply. Marbut[3] has suggested that in regions of low rainfall soil characteristics may be produced similar to those of more humid areas if the rain is concentrated in certain seasons. Also Nikiforoff (*26*) has pointed out that the surface layer of the Desert soils dries quickly after rains and may form a mulch to keep the subsoil moisture from evaporating.

In these soils the field evidence is not altogether clear as to the relationship of the surface horizon to the rest of the profile, but it is certain that some movement of the surface material has taken place. Water has occasionally flowed over the soil, but most of the time the bare surface, protected only by scattered, scrubby vegetation, is exposed to the ravages of the desert winds. Apparently the composition of the colloids has not been appreciably altered by any transfer that has taken place. No sharp break in composition occurs in the profiles, and each horizon is apparently related to its neighbor. The colloids may, therefore, be examined as in normal soil profiles.

The colloid composition varies little with the intensity of claypan development in both the residual soil profiles derived from granite and the alluvial fan soils. As sodium is low, it is evident that the colloids are saturated with other bases, the carbonates have not been removed from the lower parts of the profiles, the soils are alkaline, and the silica lost has probably been removed as a sodium silicate.

The formula weight ratios (table 4) show a certain uniformity throughout this group of Mojave Desert profiles. The silica-sesquioxide ratios ranged from 2.46 to 3.27. The difference was less in any one profile. If this ratio is corrected for the free iron oxide present in profiles A and D, it is found to be slightly higher in each horizon, ranging from 2.6 to 3.4. It usually increased in the deeper, less weathered parts of both the residual and alluvial profiles. If, however,

[3] C. F. Marbut. Soil genesis and classification. U. S. Dept. Agr. Grad. School Lectures No. 10 (6 pp.) and No. 30 (32 pp.). 1928. [Mimeographed.]

Profile and sample No.	Horizon	Depth	SiO_2	Fe_2O_3	Al_2O_3	MgO	CaO	K_2O	Na_2O	TiO_2	MnO	P_2O_5	SO_3	Ignition loss	Total	Organic matter	CO_2	N	Soluble salts	pH[2]
DERIVED FROM GRANITE																				
Profile A:		*Inches*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*P.p.m.*	
C3419	1	0-2	65.10	4.19	18.43	1.08	2.67	2.37	2.73	0.71	0.10	0.24	0.00	2.22	99.84	0.50	0.00	0.05	340	7.4
C3420	2	2-5	62.05	6.32	18.72	1.91	2.08	2.48	2.22	.70	.08	.15	.00	3.61	100.32	.25	.00	.05	140	7.4
C3421	3	5-11	62.05	6.42	18.72	1.57	2.30	2.38	2.10	.75	.13	.15	.00	3.65	100.22	.32	.00	.03	<140	7.6
C3422	4	11-14	61.10	6.98	18.33	1.51	2.95	2.28	1.99	.77	.11	.31	.00	3.88	100.21	.18	.27	.03	530	8.4
C3423	(3)	14-34	67.58	3.29	17.09	.85	2.95	3.17	2.40	.47	.08	.21	.00	1.47	99.56	.08	.00	.02	210	8.2
C3424	(3)	44-50	71.77	2.10	14.45	.69	3.90	2.24	2.42	.48	.05	.15	.00	1.35	99.60	…	.47	…	530	8.9
Profile B:																				
C3410	1	0-7	67.85	4.20	15.30	1.07	2.92	2.78	2.78	.61	.11	.10	.11	2.36	100.19	.08	.53	.02	320	8.4
C3411	2	7-10	66.70	4.82	15.53	1.59	3.16	2.72	2.40	.59	.09	.12	.00	2.71	100.43	.24	.59	.03	530	8.3
C3412	3	10-14	62.05	4.98	14.70	1.14	6.13	2.97	2.10	.53	.11	.15	.00	5.70	100.56	.31	3.11	.03	530	8.3
C3413	4	14-24	63.40	3.67	12.82	.75	7.77	2.40	2.28	.43	.07	.16	.04	6.32	100.11	.19	4.53	.03	210	8.6
Profile C:																				
C3401	1	0-6	65.36	4.03	17.80	1.18	2.40	3.33	2.26	.49	.06	.21	.01	2.51	99.64	.36	.00	.03	320	8.0
C3402	2	6-10	65.11	4.88	17.05	1.35	2.45	3.21	2.35	.66	.07	.17	.00	2.56	99.86	.29	.00	.02	320	8.0
C3403	3	10-17	61.40	5.44	16.68	1.45	4.16	2.92	2.28	.81	.08	.22	.00	4.21	99.65	.24	1.34	.02	320	7.9
C3404	4	17-26	61.59	5.88	17.33	1.50	3.88	2.84	2.72	1.05	.07	.31	.00	2.44	99.61	.16	.43	.02	420	8.1
C3405	(3)	36-48	60.08	4.00	15.18	1.06	7.35	2.52	2.82	.75	.07	.28	.00	5.50	99.61	.08	4.47	.01	210	8.2
DERIVED FROM ALLUVIAL FAN MATERIALS																				
Profile D:																				
C3395	1	0-3	69.54	3.85	14.20	0.94	2.13	3.29	2.76	0.50	0.06	0.13	0.02	2.49	99.91	0.66	0.00	0.04	460	8.1
C3396	2	3-12	70.23	3.76	14.45	.79	1.53	3.45	2.89	.46	.05	.12	.00	1.81	99.54	.24	.00	.03	140	7.8
C3397	3	12-24	66.67	5.12	15.85	1.07	1.66	3.20	2.28	.57	.06	.10	.00	2.98	99.56	.24	.00	.03	180	7.4
C3398	4	24-30	66.41	5.21	15.84	1.19	2.01	2.97	2.65	.53	.06	.08	.00	2.91	99.86	.17	.00	.03	210	7.8
C3399	5	30-36	68.06	4.71	15.42	1.13	2.17	2.88	2.49	.58	.06	.10	.00	2.43	100.03	.16	.00	.02	320	8.3
C3400	6	36-50	70.36	3.66	14.40	.94	2.07	3.21	2.76	.50	.05	.07	.00	1.79	99.81	.13	.00	.02	320	8.5
Profile E:																				
C3414	1	0-5	68.81	3.59	15.55	.76	1.74	3.23	3.06	.47	.19	.15	.00	2.38	99.93	.67	.00	.07	140	7.9
C3415	2	5-10	67.30	4.41	16.27	1.11	1.71	3.10	2.86	.57	.15	.11	.01	2.70	100.33	.26	.00	.03	140	7.7
C3416	3	10-20	67.35	3.82	17.28	1.09	1.52	2.57	2.06	.55	.07	.20	.00	3.15	99.65	.20	.00	.03	<140	7.6
C3417	4	20-34	65.70	4.21	17.00	1.20	2.57	2.44	2.33	.57	.06	.23	.00	3.37	99.68	.15	.44	.02	340	8.5
C3418	5	34-44	71.00	3.63	15.89	.93	1.84	2.47	2.51	.42	.06	.12	…	1.56	100.43	…	…	…	340	8.5
Profile F:																				
C3879	1	0-3	72.69	2.78	13.39	.72	2.15	2.79	2.37	.50	.04	.19	.00	1.80	99.42	.43	.00	.03	250	7.8
C3880-81	[4] 2-3	3-16	71.29	3.29	14.61	.90	2.30	2.66	2.02	.52	.05	.17	.00	1.88	99.69	.18	.00	.01	155	7.9
C3882-84	[4] 4-5-6	16-48	69.35	3.33	13.79	1.06	3.77	2.48	2.02	.50	.06	.15	.00	2.75	99.26	.12	.93	.01	320	8.5
C3885	7	48-60	61.47	4.87	15.65	2.15	5.40	2.21	2.21	.80	.06	.17	.00	4.87	99.86	.16	1.69	.01	550	8.7

[1] Organic matter by the combustion method (CO_2×0.471), CO_2 of the carbonates, and N determined by J. H. Shimp.
[2] pH values determined by E. H. Bailey.
[3] Rotten rock.
[4] Composited.

TABLE 3.—*Chemical analyses of Mojave Desert soil colloids* [1]

Profile and sample No.	Horizon	Depth	Colloid extracted	SiO_2	Fe_2O_3	Al_2O_3	MgO	CaO	K_2O	Na_2O	TiO_2	MnO	P_2O_5	SO_3	Ignition loss	Total	Organic matter	CO_2	N
		Inches	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
DERIVED FROM GRANITE																			
Profile A:																			
C3419	1	0-2	24	44.95	10.74	24.24	3.01	1.21	2.44	0.15	0.88	0.17	0.44	0.08	11.54	99.85	3.41	0.00	0.32
C3420	2	2-5	30	47.50	10.39	25.37	2.91	1.13	1.99	.03	.97	.19	.16	.00	9.47	100.11	.61	.00	.09
C3421	3	5-11	33	48.00	11.14	24.19	2.97	1.28	1.67	.00	.95	.11	.12	.00	9.49	99.98	.64	.00	.05
C3422	4	11-14	30	49.49	10.82	22.50	3.30	1.80	.87	.15	.87	.04	.20	.00	9.41	99.45	.50	.09	.08
C3423	5	14-34	6	50.12	9.70	22.04	3.41	2.51	.70	.13	.78	.08	.20	.00	9.96	99.63	.82	.27	.09
Profile B:																			
C3410	1	0-7	18	49.30	10.07	21.72	3.45	2.02	2.81	.21	.85	.12	.38	.05	9.40	100.38	1.58	.02	.17
C3411	2	7-10	27	51.45	9.40	21.70	3.84	1.90	2.06	.27	.70	.06	.14	.02	8.71	100.25	.79	.00	.10
C3412	3	10-14	26	48.35	8.68	20.42	3.70	5.49	1.75	.21	.61	.05	.09	.02	11.30	100.67	.29	3.56	.10
C3413	4	14-24	28	46.30	8.70	19.02	3.39	6.48	1.42	.19	.72	.05	.38	.01	13.23	99.95	.36	4.73	.11
Profile C:																			
C3401	1	0-6	18	50.61	9.15	22.36	3.35	2.45	1.20	.22	.72	.08	.37	.03	9.20	99.82	1.17	.00	.11
C3402	2	6-10	12	51.30	9.24	21.23	3.50	2.43	1.36	.09	.73	.04	.22	.00	9.35	99.49	.96	.00	.09
C3403	3	10-17	15	47.44	7.86	20.83	3.28	6.35	1.18	.04	.68	.04	.28	.00	11.88	99.86	.86	3.09	.08
C3404	4	17-26	11	49.09	9.15	21.40	3.03	4.16	1.06	.09	.68	.04	.35	.00	10.82	99.87	1.28	1.18	.11
DERIVED FROM ALLUVIAL FAN MATERIALS																			
Profile D:																			
C3395	1	0-3	21	51.07	8.90	20.90	3.44	1.83	1.98	0.43	0.52	0.16	0.12	0.02	10.42	99.85	2.81	0.00	0.28
C3396	2	3-12	32	49.28	9.25	24.36	2.68	1.31	2.74	.17	.51	.08	.14	.00	8.96	99.48	1.20	.00	.13
C3397	3	12-24	29	49.17	9.80	24.10	2.65	1.45	1.36	.11	.58	.06	.12	.00	10.02	99.42	.82	.00	.10
C3398	4	24-30	29	49.50	10.06	24.08	2.73	1.77	1.09	.26	.59	.06	.06	.00	9.84	100.04	.92	.00	.12
C3399	5	30-36	23	50.55	10.12	23.39	2.79	1.93	1.16	.18	.57	.08	.06	.00	9.39	100.22	.82	.00	.11
C3400	6	36-50	22	51.37	10.18	21.81	3.20	2.01	1.44	.18	.50	.08	.14	.00	8.71	99.62	.96	.00	.10
Profile E:																			
C3414	1	0-5	26	49.30	10.63	23.17	3.06	1.53	2.62	.29	.69	.15	.29	.03	9.13	100.89	1.35	.00	.14
C3415	2	5-10	32	50.20	10.41	23.47	2.91	1.41	2.52	.10	.68	.08	.16	.08	8.50	100.52	.82	.00	.09
C3416	3	10-20	61	50.42	9.69	23.33	2.95	1.32	1.77	.02	.70	.09	.08	.01	9.14	99.52	.52	.00	.06
C3417	4	20-34	40	50.45	10.06	22.50	3.08	1.81	1.63	.06	.87	.07	.12	.00	9.51	100.16	.64	.00	.08
C3418	5	34-44	------	50.80	10.22	22.20	3.22	1.92	1.62	.11	.67	.09	.21	.00	9.07	100.13	.68	.00	.09
Profile F:																			
C3879	1	0-3	------	46.27	9.40	23.14	3.46	1.83	2.33	.26	.44	.08	.24	.00	13.12	100.57	4.20	.08	.38
C3880-81	[2] 2-3	3-16	------	48.94	8.96	23.86	3.47	1.84	2.51	.25	.44	.05	.18	.00	9.48	99.98	1.24	.02	.13
C3882-84	[2] 4-5-6	16-48	------	49.11	7.79	22.03	3.88	3.86	2.48	.25	.38	.06	.18	.00	10.83	100.85	.89	1.65	.14
C3885	7	48-60	------	48.19	8.01	20.19	4.46	5.15	1.72	.28	.36	.06	.22	.00	11.15	99.79	1.00	2.51	.11

[1] Organic matter determined by the combustion method ($CO_2 \times 0.471$). CO_2 of the carb...

[2] Composited.

·on oxide, whether free or combined, is wholly ignored and silica is ompared directly with alumina, it is noted that about the same niformity prevails throughout the samples, the ratios ranging from .16 to 4.15. With few exceptions a fairly regular increase with depth ccurred irrespective of the amount of clay in the profile. Although he change was not great, it indicated that the small amount of water hat penetrated the soils had removed silica from the upper parts of 10st of the profiles.

TABLE 4.—*Derived data: Mojave Desert colloids*

DERIVED FROM GRANITE

·rofile and sample No.	Horizon	Depth	Formula ratio $SiO_2/Fe_2O_3.Al_2O_3$	Formula ratio SiO_2/Fe_2O_3[1]$.Al_2O_3$	Formula ratio SiO_2/Al_2O_3	Formula ratio $SiO_2/$Total bases[2]	Formula ratio SiO_2/H_2O[3]	Formula ratio H_2O[3]$/Al_2O_3$	Combined water [4]	Combined water of the soil acid [5]
rofile A:		*Inches*							*Percent*	*Percent*
C3419	1	0–2	2.46	2.6	3.16	6.01	1.30	2.43	8.13	10.64
C3420	2	2–5	2.52	2.7	3.18	6.94	1.30	2.71	8.86	10.98
C3421	3	5–11	2.61	2.8	3.52	6.94	1.32	2.83	8.85	11.00
C3422	4	11–14	2.86	3.0	3.74	6.56	1.33	2.82	8.91	11.24
C3423	5	14–34	3.02	3.2	3.86	6.65	1.35	2.86	8.77	11.31
rofile B:										
C3410	1	0–7	2.98	3.1	3.85	5.30	1.34	2.87	8.20	11.17
C3411	2	7–10	3.16	3.3	4.03	5.52	1.44	2.80	7.92	10.80
C3412	3	10–14	3.15	3.3	4.01	4.96	1.39	2.88	7.45	11.16
C3413	4	14–24	3.19	3.4	4.12	5.07	1.40	2.94	7.14	11.52
rofile C:										
C3401	1	0–6	3.05	3.2	3.85	5.86	1.41	2.73	8.17	10.88
C3402	2	6–10	3.21	3.4	4.10	5.82	1.39	2.95	8.39	11.14
C3403	3	10–17	3.12	3.3	3.87	5.72	1.34	2.89	8.13	11.52
C3404	4	17–26	3.06	3.2	3.85	6.03	1.36	2.83	8.36	11.26
DERIVED FROM ALLUVIAL FAN MATERIALS										
rofile D:										
C3395	1	0–3	3.26	3.4	4.15	5.83	1.39	2.99	7.61	10.57
C3396	2	3–12	2.76	2.9	3.42	6.72	1.45	2.36	7.76	10.08
C3397	3	12–24	2.75	2.9	3.40	7.60	1.32	2.62	9.20	11.24
C3398	4	24–30	2.75	2.9	3.49	7.14	1.35	2.59	8.92	11.11
C3399	5	30–36	2.88	3.0	3.67	6.91	1.21	3.03	8.57	12.67
C3400	6	36–50	3.07	3.2	3.99	6.64	1.28	3.12	7.79	12.07
rofile E:										
C3414	1	0–5	2.78	2.9	3.60	6.04	1.43	2.52	7.78	10.41
C3415	2	5–10	2.72	2.9	3.45	6.66	1.52	2.27	7.62	9.97
C3416	3	10–20	2.90	3.1	3.67	7.26	1.41	2.60	8.62	10.76
C3417	4	20–34	2.96	3.1	3.81	6.62	1.24	3.07	8.87	12.24
C3418	5	34–44	3.00	3.2	3.87	6.36	1.41	2.75	8.39	10.85
rofile F:										
C3879	1	0–3	2.70	2.9	3.39	5.20	1.21	2.80	8.84	11.95
C3880-81	[6] 2–3	3–16	2.77	2.9	3.43	5.48	1.34	2.56	8.22	11.05
C3882-84	[6] 4–6	16–48	3.08	3.2	3.78	5.18	1.32	2.86	8.29	11.66
C3885	7	48–60	3.27	3.4	4.10	4.86	1.37	2.99	7.64	11.44

[1] Combined iron oxide.
[2] Oxides of Mg, Ca, K, and Na.
[3] Ignition loss less the organic matter, plus the water equivalent of the bases.
[4] Ignition loss less the organic matter.
[5] Ignition loss less the organic matter plus the water equivalent of the bases, corrected for the organic ıatter and carbonate content.
[6] Composited.

The silica-base ratio was low and showed only slight variation in ·ach profile as well as in the whole group of samples, irrespective of he origin of the materials on which the soils had formed. Although ·emoval of bases, especially sodium, had occurred during the hylrolysis and degradation of the primary minerals, a large part of the nagnesium and most of the other bases still remained in the colloid.

The combined water in the whole group of colloids ranged from 7.14 to 9.20 percent. The average was 8.30. The variation from the mean was 18.9 percent. In profiles B and F the combined water was higher in the surface horizon than in other parts. In the other profiles the combined water tended to be greater in the deeper, cooler parts. In none of the profiles did it follow closely the clay content of the soils.

The combined water of the soil acid postulated by Byers (*7*) showed less variation than the combined water alone. The variation from the mean was 13.4 percent, and the tendency to increase with depth occurred more regularly and more often than in the combined water. The highest values always occurred in the deeper parts of those profiles containing a claypan. The hypothetical combined water of the soil acid is obtained by adding the water equivalent of the bases to the combined water and correcting the sum for organic matter. It carries no implication as to how the various constituents are combined in the component clay minerals of the soil.

A comparison of either silica or alumina with the combined water of the soil acid shows still greater uniformity than the other ratios throughout all of the profiles. The average of the ratio of the combined water of the soil acid to silica was 1.35 and to alumina, 2.77. The variation from the mean of the first was 11.5 percent and of the second, 12.6. The variation within any one profile was very much less. These ratios indicate a remarkable uniformity in the relationships between the three principal constituents of the colloids irrespective of the amounts of each in the component minerals in the colloid.

The calculation of the combined water of the soil acid rests on the assumption that all of the bases present are replaceable by hydrogen on complete hydrolysis of the colloid complex. Recent evidence based on X-ray examinations indicates that a part of some of the bases, particularly magnesium, exists as a substituent for aluminum in the lattice structure of the clay minerals (*14*, *24*, *25*, *28*). Consequently the assumption that hydrogen can replace all of the bases is probably not valid. Moreover, the base-exchange relations of the colloids (table 5) show that only about 11 percent of the total magnesium is exchangeable. The work of Kelley, Dore, and Brown (*19*) indicates that, upon grinding, all of the bases of the colloids can be extracted with ammonium acetate. This does not necessarily show that an increase in the base-exchange capacity occurs and that these bases are actually replaceable by hydrogen. Probably this increase of bases, removed after grinding, is accompanied by the disintegration of a part of the colloid complex, as is indicated by Drosdoff (*10*).

These various relationships indicate that the principal constituents of the colloids are essentially the same in all the profiles presented. It is also evident that profile variations of the colloid composition are slight irrespective of the remarkable quantity of clay in parts of most of the profiles. It appears certain, however, that the composition of the colloid is only slightly influenced by many factors that cause profound differences in the distribution of the clay and other morphological properties of the soils. The changes in morphology are greater than expected in soils receiving so little rainfall.

Another point of interest is the fact that the colloid extracted from the rotten granite rock of profile A, horizon 5, has essentially the same chemical and mineralogical composition as the claypan horizon containing over 35 percent of clay. It was surprising to find that the apparently slightly weathered granite contained as much as 14 percent of clay. As the material had been broken and powdered in the course of collecting the sample, a further mechanical analysis was not made, but from observation it appeared that the material was largely coarse sand and gravel with the clay particles sticking to the coarser particles. Although rock decomposition in this profile is far from complete, apparently the colloids formed have reached an equilibrium with their environment.

Information about the base status of representative colloids was obtained by determining the exchangeable-base and base-exchange capacity of one of the residual soils (profile A) and one of the alluvial fan soils (profile D). These results are shown in table 5. They indicate that the colloids are practically all saturated with bases and that those which are replaceable consist almost entirely of calcium and magnesium. The exchangeable sodium and potassium were very low. In profile A the ratio of exchangeable calcium to exchangeable magnesium was approximately 2 to 1 whereas in profile D it was roughly 4 to 1.

The calcium present in the colloids was over 83 percent exchangeable in all except the lowest layer in each profile. It appears that the horizons having the most clay have less calcium in the nonexchangeable form. This indicates that hydrolysis has proceeded further in the claypan than in other layers. Only about 10 to 15 percent of the total magnesium, and a very small fraction of the potassium, are apparently present in the exchangeable form. These bases, therefore, must be firmly bound and must be present largely as a more permanent part of the clay minerals.

TABLE 5.—*Replaceable bases in colloids*

PROFILE A

Laboratory No.	Horizon	Ca		Mg		Na		K		Total replaceable bases	Base-exchange capacity
		Milliequivalents per 100 gm.	Percent of total CaO	Milliequivalents per 100 gm.	Percent of total MgO	Milliequivalents per 100 gm.	Percent of total Na_2O	Milliequivalents per 100 gm.	Percent of total K_2O	Milliequivalents per 100 gm.	Milliequivalents per 100 gm.
C3419	1	37.2	86	22.5	15	1.2	20	0.2	1	61.1	-------
C3420	2	36.5	90	15.6	11	.9	100	.3	1.5	53.3	-------
C3421	3	43.9	96	24.4	16	1.0	50	.3	2	69.6	-------
C3422	4	56.2	87	22.1	13	.9	20	.2	2	79.4	-------
C3423	5	47.0	53	19.3	11	-------	-------	-------	-------	66.3	-------

PROFILE D

Laboratory No.	Horizon	Ca milliequivalents per 100 gm.	Ca percent of total CaO	Mg milliequivalents per 100 gm.	Mg percent of total MgO	Na milliequivalents per 100 gm.	Na percent of total Na_2O	K milliequivalents per 100 gm.	K percent of total K_2O	Total replaceable bases	Base-exchange capacity
C3395	1	55.2	84	11.6	7	1.0	8	0.3	1.5	68.1	74.3
C3396	2	40.3	87	12.2	9	-------	-------	-------	-------	52.5	57.7
C3397	3	50.4	97	13.8	10	.8	20	.2	1.5	65.2	67.3
C3398	4	62.4	99	13.6	10	.7	10	.3	3	77.0	75.3
C3399	5	60.5	87	13.9	10	.9	16	.9	6	76.2	77.4
C3400	6	56.3	79	14.1	9	.8	11	.8	6	72.0	78.3

In order to obtain more definite information on the clay mineral content of the colloids, X-ray analyses were made of those from profiles A and D.[4] The clay minerals present were found to be essentially the same in all of the samples and consisted of about 25 percent of kaolinite (halloysite) and 70 to 80 percent of what can best be described as a mixed-layer mineral. This type of clay mineral has been discussed by Hendricks and Alexander (*15*). It was shown by Alexander, Hendricks, and Nelson (*1*) to be a composite mineral of montmorillonite and hydrous mica. That such minerals can be formed by interstratification of different kinds of layers has been shown by Gruner (*13*). Though this type of mixed mineral is difficult to recognize, it is probably a common constituent of soils (*15*).

The montmorillonite layers are essentially the same in composition as the hypothetical "pyrophillic acid" postulated by Byers (*7*). The ideal formula has a silica-alumina ratio of 4 to 1 and is modified by isomorphous replacements, as discussed later.

The hydrous mica type of clay mineral has recently been recognized as a common constituent of soil colloids by a number of investigators (*11, 12, 23*). The ideal formula may be considered to be the same as that given for mica but with less potassium and more water. The nonexchangeable potassium is firmly bound in the crystal lattice of the hydrous mica. It has been pointed out that the percentage may vary considerably without changing the structure of the mineral (*15*). In the colloids examined here, the potassium oxide content ranged from 0.70 to 2.74 percent, whereas the amount of the mixed-layer mineral, hydrous mica-montmorillonite, remained approximately the same.

The nonexchangeable magnesium of the colloid may be present in either the hydrous mica or the montmorillonite layers. The variation in the amount depends on how much isomorphous replacement of magnesium for alumina occurs. That magnesium can replace aluminum in the lattice structure in the ratio of 3 of magnesium to 2 of aluminum is now generally accepted (*14, 24, 25, 28*). The combined iron may also substitute for aluminum in the internal structure of the clay mineral.

As stated above, about 25 percent of the colloids of these soils is composed of kaolinite or halloysite, which is similar in composition to the hypothetical "halloysitic acid" postulated by Byers (*7*). The ideal composition has a silica-alumina ratio of 2 to 1. There is very little isomorphous replacement in this type of clay mineral, since it is largely restricted to mutual substitutions of aluminum and silica. Apparently the X-ray data indicate that kaolinite and halloysite are very similar and belong to the family of kaolin minerals (*30*).

The reddish-brown color of the colloids indicates the presence of some free iron oxide. The amount was determined by the hydrogen sulphide method described by Truog et al. (*32*) on the colloids of soils A and D. The results are presented in table 6. It was found that about one-fourth of the iron near the surface, and about one-fifth in deeper parts of the profile, is free oxide; the rest is combined as a silicate. As colloids from profiles A and D are similar to the others in composition, approximately the same amount of free iron oxide is

[4] These analyses were made by S. B. Hendricks, of the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture.

assumed to be present in the colloids of comparable parts of the other profiles. Reddish-brown soils of the humid regions have a much larger proportion of free iron oxide. Therefore it appears that under conditions of low rainfall the weathering processes result in the segregation of a comparatively small amount of free iron oxide, most of the iron remaining in the combined form. The fact that approximately one-fourth of the total iron is present as free oxide suggests that its formation is coincident with the formation of kaolinite, as this clay mineral comprises about one-fourth of the colloid. The kaolin minerals do not contain appreciable amounts of iron or magnesium.

TABLE 6.—*Percentage of iron oxide in the colloids*

Laboratory No.	Horizon	Depth	Total	Free	Proportion free
PROFILE A, DERIVED FROM GRANITE					
		Inches	*Percent*	*Percent*	*Percent*
C3420	2	2–5	10.39	2.8	27
C3421	3	5–11	11.14	2.2	20
C3422	4	11–14	10.82	2.4	22
C3423	5	14–34	9.70	2.0	21
PROFILE D, DERIVED FROM ALLUVIAL FAN MATERIALS					
C3396	2	3–12	9.25	2.6	28
C3397	3	12–24	9.80	2.0	20
C3398	4	24–30	10.06	2.1	21
C3400	6	36–50	10.18	1.9	19

Aside from the predominant mixed-layer mineral, hydrous mica-montmorillonite, the kaolinite, and the small amount of free iron oxide as determined by chemical analysis, there is also the doubtful possibility of a small percentage of quartz present. Quartz and free iron oxide are present in amounts too small to be detected with certainty by X-ray analysis.

It is interesting to note that the coarse biotite-vermiculite mica particles of the partially weathered granite of the residual soils gave essentially the same X-ray pattern as the predominant clay mineral of the colloid. This suggests that the micas are more important in the formation of clay from granite than has been previously supposed. It has been generally assumed that because the feldspars are by far the most predominant mineral group in granite, the clay minerals are formed largely from them, either directly or indirectly. The coarse mica in the rock contains 5.5 percent of potassium oxide and 7.1 percent of combined water. With further hydrolysis, potash would be replaced by water and thereby approach the composition of the soil colloid. Denison, Fry, and Gile (*9*) determined the changes in composition of the coarse mica particles undergoing weathering and with mineralogical control showed a continuous variation from the pure micas to the highly weathered micas, which are similar to the hydrous mica of the soil colloid.

The laboratory data presented corroborate the suggestion of Nikiforoff (*26*) that the claypan in the Mojave Desert soils is formed in place by hydrolysis of the primary minerals of the soil. Evidence of this is found in the remarkable uniformity of the chemical and mineral-

ogical composition and the amount of degradation of the colloids derived from both residual granite and alluvial fan materials in the region of extremely low rainfall. The coarse mica is of the same composition as the predominant clay mineral colloids of the soil. The alluvial fan profiles appear to be as well developed as the residual granite and do not reflect the conditions of deposition. Profile development occurs without significant change in the colloids. This indicates that the claypan is developed in place and is genetically related to the other horizons. The amount of clay in any horizon is dependent on the moisture in the soil, the age of the soil, and the freedom of the soil from erosion.

SUMMARY

Six soil profiles from the Mojave Desert have been studied. Of these, three are residual soils derived from granite; three are derived from alluvial fan material; five have well-developed claypan horizons, and one has none. They are from a comparatively restricted area near Mojave, Calif., and occur at 2,700 to 3,000 feet above sea level. The mean annual rainfall presumably is approximately 5 inches and the mean annual temperature is about 65° F.

The results show that the chemical alteration of claypan soils is greater than the moisture supplied by the rainfall of the desert is expected to produce. Neither the chemical nor the mineralogical composition of the colloids is influenced by the formation of a claypan in these desert soils. The colloids are saturated with bases, chiefly calcium and magnesium. Of the total iron, 25 percent is present as free iron oxide.

The X-ray data indicate that about 75 percent of the colloids is composed of a mixed-layer mineral of hydrous mica and montmorillonite similar to the coarse weathered mica in the soil. About 25 percent is kaolinite (halloysite).

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EFFECT OF FEEDING REPEATED SMALL DOSES OF SELENIUM AS SODIUM SELENITE TO EQUINES[1]

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INTRODUCTION

Much loss has been occasioned to the livestock industry through chronic selenium poisoning in a number of sections of the north-central Great Plains. Animals fed grain and roughage grown in these areas manifest, after varying lengths of time, a more or less characteristic syndrome known as alkali disease. The symptoms most frequently described are unthriftiness, loss of weight and hair, and abnormal growth of the horn of the hoof with attendant lameness. In poultry, failure to reproduce because of poor hatchability of eggs or the occurrence of weak, deformed chicks, has also been observed. Considerable uncertainty existed as to the cause of this condition until about 7 years ago, when selenium was definitely associated with the disease.

Since that time many experiments have been carried out to test the action of selenium on various species of animals. Both seleniferous feed and different organic and inorganic compounds of selenium have been used for this purpose. As a consequence, much information has been obtained on the toxicity and pathological effect of selenium on laboratory animals, such as rats, rabbits, cats, and dogs, and in a few instances on the larger domestic animals. Two experiments have been reported in which swine were the test animals. In one of these (*6*),[2] seleniferous corn grown in affected areas was fed; in the other (*3*), sodium selenite was added to the feed to give different concentrations of selenium. Typical symptoms of selenium poisoning were produced in some of the animals in each of these experiments. In the light of the results of this work and since there is no known information on the experimental feeding of repeated small doses of selenium to equines, it was decided to investigate this phase of the problem. The work was done during 1937 and 1938 at the Animal Disease Station, Beltsville, Md.

EXPERIMENTAL PROCEDURE

Because of the limited number of animals available for the experiment, approximately the same concentration of selenium was given to each animal. In the beginning of the experiment, two horses and a mule were used, and some time later a third horse was added. Of the three horses, one (No. 962) was a mare about 12 years old, the second (No. 980) was a gelding about 5 to 6 years old, and the third (No. 1023) was an aged gelding which had been used previously in an equine encephalomyelitis experiment. The mule, a gelding (No. 1034), was also an aged animal which had passed through other disease experiments. However, all the animals were apparently in good health when this experiment began.

[1] Received for publication December 29, 1939.

[2] Italic numbers in parentheses refer to Literature Cited, p. 368.

About 5 weeks before the selenium was added to the feed, horses 962 and 980 and the mule were placed in a small stable with four stalls and an exercise yard about 100 feet square. There were a few weeds and several shade trees in the yard but no vegetation for the animals to eat. The animals were watered only when they were turned out for exercise, which was from 9 a. m. to 4 p. m. daily. The water was obtained from the regular supply at the Animal Disease Station at Beltsville, Md.

The feed, consisting of No. 2 oats and No. 2 timothy hay, was obtained from the station supply, which was used in feeding all the horses kept on the premises, both for work and experimental purposes. After some experimentation, it was found that 5 pounds of oats with 20 pounds of hay for the horses and 5 pounds of oats with 15 pounds of hay for the mule were adequate rations which would be cleaned up regularly. These rations were continued for the duration of the experiment. When the third horse was added, it received 5 pounds of oats and 15 pounds of hay. Any oats and hay left in the mangers following the addition of sodium selenite to the feed were weighed after each feeding.

At the beginning of the experiment, selenium in the form of sodium selenite was given at the rate of 24 parts per million of feed to the horses and 17.3 parts per million to the mule. This proportion was obtained by mixing 35 cc. of an M/10 solution of sodium selenite (17.3 gm. per liter) in distilled water with the 5 pounds of oats for the horses; 21 cc. of the solution was given to the mule in the same manner. The animals ate the selenized oats readily at first but after about 2 months refused them and ate only hay. The method of administration was then changed to drenching each animal with the sodium selenite solution by means of a dose syringe after the oats had been eaten in the morning. Hay was fed at night while the animals were in the stable. To facilitate drenching, an M/5 solution of sodium selenite (34.6 gm. per liter) was made up and the volume of the dose reduced by half, namely, 17.5 cc. to the horses and 10.5 cc. to the mule. This method was continued for about 4 months, making a total of 6 months that the horses ingested selenium equivalent to 24 parts per million of feed and the mule, 17.3 parts. Then the dose was doubled with the result that each horse received 48 parts, and the mule 34.6 parts, of selenium per million parts of feed, 35 cc. of an M/5 solution of sodium selenite being given to the horses and 21 cc. to the mule.

About 4 months later another change was made in the method of administering the selenium to the horses, the mule having died in the meantime. This was necessitated by the fact that both of the horses gradually refused to swallow the solution and part of it was lost at each drenching. This difficulty was overcome by diluting the daily dose of M/5 solution of sodium selenite to 700 cc. with water. Of this diluted solution, 150 cc. was sprinkled on the oats and the remainder on the hay. In order to obtain an even distribution of the solution in the hay, as well as to facilitate weighing, the hay was chopped into lengths of about 1½ inches. The horses ate the chopped hay as well as when it was in its original form, and they continued to eat both the selenized oats and hay satisfactorily until shortly before death. Since the condition of both horses seemed to have remained relatively static even after they had ingested 48 parts of selenium per million of feed for

about 9 months, the dose was increased to about 96 p. p. m., 70 cc. of an M/5 solution of sodium selenite being diluted to 700 cc. with water and then mixed with the feed as previously described. This quantity was given until the experiment was terminated by the death of the animals.

About a month after the last increase in the dose of selenium, horse 1023 was placed on experiment. It was given the same dose of sodium selenite solution as the other two horses, although it was eating 5 pounds less hay. The selenium level for this animal was, therefore, approximately 115 parts per million of feed. Three weeks later the concentration was reduced to 96 p. p. m. by giving 56 cc. of the M/5 solution of sodium selenite diluted to 700 cc. with water. This dose was continued for 2 weeks, or until the horse died.

In order to determine what concentration of selenium might be produced in the blood by feeding repeated small doses of selenium, samples of blood were taken from each animal before the experiment began and each week thereafter. The blood was taken from the jugular vein and at approximately the same time of day on each occasion. Fecal and urine samples were collected at irregular intervals and at the same time as the blood samples. The feces were removed directly from the rectum. Urine was obtained from the mare (No. 962) by means of a catheter. The first few urine samples were drawn from the geldings (Nos. 980 and 1034) by passing the catheter, but later it was found that the animals could be induced to urinate by brisk massage of the bladder and the urine was caught in a clean pan. In addition to the analysis of these specimens, selenium determinations were made on the body tissues and fluids which were collected at the time of autopsy.

EXPERIMENTAL RESULTS

HORSE 962

Horse 962 received a small dose of selenium daily for a period of 17 months and 12 days before death occurred. At the beginning of the selenium-feeding period (July 26, 1937) the animal weighed 1,000 pounds, whereas at the time of death its weight had decreased to 660 pounds. The weekly changes in the weight of the animal are shown in figure 1. Although there was considerable fluctuation in the weight from week to week, a definite downward trend did not appear until about 3½ months after the experiment began. From that time on there was a gradual decrease until 3 weeks before death, when the weight decreased abruptly. The animal's appetite remained good until the last 10 days of the experiment, when practically all the oats and hay were left untouched.

Although the level of selenium given in the feed to this animal was relatively high and had been found to be effective in producing typical symptoms of chronic selenium poisoning in swine (*3*), pronounced physical changes such as have been described in horses grazing on seleniferous soil (*2*) were not observed. The symptoms, other than emaciation and listlessness, were rather mild. Figure 2, *A*, shows horse 962 as it appeared 1 week after the beginning of the experiment, and *B*, the same animal 3 weeks prior to death, or about 17 months later. Up to that time the appetite had been good, but there had been a loss of about 150 pounds in weight, the coat was staring, and

when the animal was aroused it moved slowly with an unsteady gait. The hair was loose in the mane and tail, particularly in the mane, which contained much matted hair. There was no evidence of loosening of the hair until about 6 months after the beginning of the experiment. From that time on, the hair could be pulled out readily but there was never any tendency for it to shed abnormally.

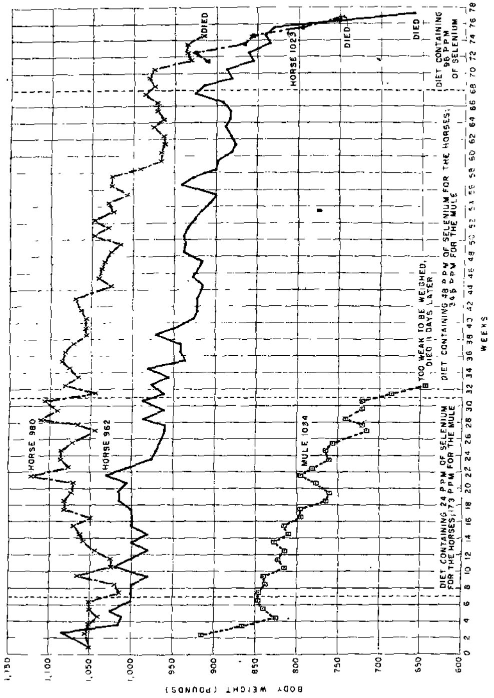
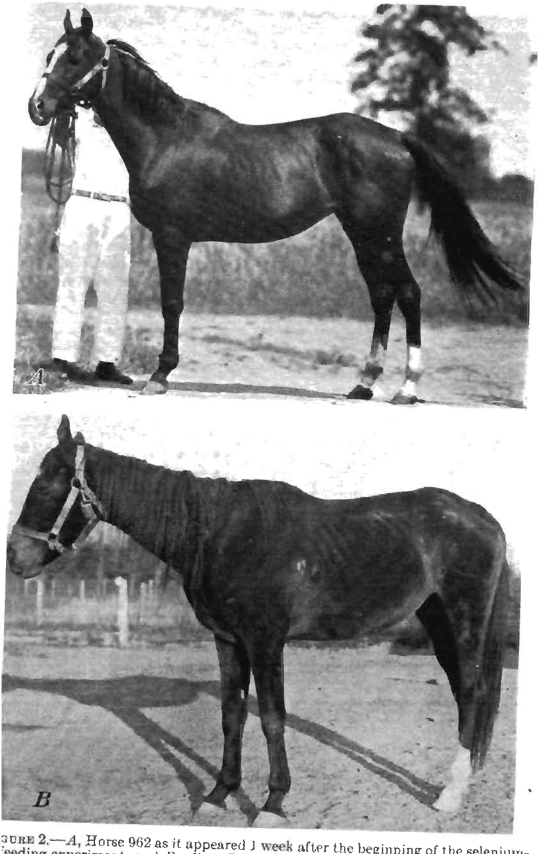


FIGURE 1.—Weekly changes in body weight of the experimental animals as a result of ingesting small repeated doses of selenium in the form of sodium selenite.

Changes in the horn of the hoofs were observed, but they were not outstanding. A year after the experiment began, there was considerable scaling of the horny wall of the hoofs for a distance of several inches below the coronary band. When the loose scales were removed, the horn had a white flaky appearance. In addition, the



GURE 2.—*A*, Horse 962 as it appeared 1 week after the beginning of the selenium-
eeding experiment, and *B*, after 17 months of selenium feeding. Note emacia-
ion, drowsiness, and matted hair of mane in *B*.

GURE 2.—*A*, Horse 962 as it appeared 1 week after the beginning of the selenium-eeding experiment, and *B*, after 17 months of selenium feeding. Note emacia-ion, drowsiness, and matted hair of mane in *B*.

horn seemed softer, as indicated by excessive wear of the hoofs. The latter condition occurred in spite of the fact that the animal was confined in a relatively small lot on soft sandy loam. Slight scaling of the wall first appeared about 3 months after the experiment began and persisted during the life of the animal. No separation of the hoof from the skin at the coronary band was ever observed. In addition to this change, the feet seemed to become sore at irregular intervals for periods of several weeks. This soreness was evidenced by the short stilted steps taken by the animal in walking and a constant shifting of the weight when standing. At such times the coronary band seemed to be slightly swollen and somewhat painful on palpation.

Another symptom which was manifested occasionally was extreme nervousness or sensitivity when blood samples were taken. As the needle was introduced into the jugular vein, the neck muscles would contract and almost shut off the flow of blood, whereas at other times the animal remained quiet and offered no resistance while being bled. One other peculiarity was observed during the course of the experiment. About 3 months after selenium feeding began, this horse, as well as horse 980 and mule 1034, was observed chewing on the wooden parts of the fence of the enclosure. Somewhat later the animals were seen licking the dirt in the yard, in spite of the fact that stock salt was accessible at all times. Other animals maintained on the same feed without selenium showed no evidence of depraved appetite.

Post-mortem examination showed the carcass of horse 962 to be in a very emaciated condition. The hair of the mane and tail was loose, and all four hoofs showed some flaking of the horn. Both the mucous membranes and the conjunctiva were pale and icteric. There was some emphysema of the lungs, due possibly to the labored terminal breathing. The fat of the heart was of a gelatinous consistency, the heart muscle was pale, and the endocardium was yellow and spotted with a few subendocardial hemorrhages. The liver was darker in color and somewhat smaller than normal. The surface of this organ showed many fibrinous tags and a prominent demarcation of the lobules, indicative of marked cirrhosis. On sectioning, the cut surface was yellowish chocolate in color and there was a marked increase in the connective tissue. A few subcapsular petechiae were present on the surface of the spleen, which was somewhat smaller than usual. When sectioned, the surface was dry, the pulp was dark and doughy, and there was an increase in the connective tissue. The kidneys were chocolate in color and the capsules were difficult to remove. A few hemorrhages were found in the cortex of the organ. The peritoneum was icteric and there was a slight enteritis.

HORSE 980

Horse 980 received the same quantity of feed and the same dose of selenium as horse 962. Although it weighed about 50 pounds more at the beginning of selenium feeding and remained slightly heavier throughout the course of the experiment, it died about 2 weeks before the latter animal. The changes in weight during the feeding period appear in figure 1. The weekly fluctuations in the weight of horse 980, as well as the general downward trend, although beginning a little later, fairly closely parallel those of horse 962. In horse 980,

however, the final precipitous decrease in weight was interrupted by the death of the animal. As in the first case, the appetite of horse 980 remained good until about 10 days prior to death.

The clinical picture was largely a replica of that of horse 962. In figure 3, *A*, horse 980 is shown as it appeared 1 week after the beginning of the experiment, and *B*, the same horse 3 days before death. By that time there had been a loss of about 200 pounds in weight and the animal was barely able to walk.

Changes in the character of the horn of the four hoofs just below the coronary band appeared about 2 months after the beginning of the experiment. A view of the front feet after the animal had been fed selenium for 1 year is shown in figure 4. There was distinct swelling of the coronary band (*a*), a roughened scaly formation on the horny wall about 1½ inches wide immediately below (*b*), and extreme wear and breaking away of the horn at the toes. A small portion of the horny wall that was present before the beginning of selenium feeding and that was distinguished from the selenized horn by a slight depression remained on the toes of both front feet. This ringed appearance was first noticed at the top of the hoofs shortly after the horn became roughened. Since the horse showed little disposition to move around a great deal from this time on, no further breakage occurred and the character of the horn showed little subsequent change. Periodic tenderness of the feet at irregular intervals was noticed in this animal also, although not always at the same time that it appeared in horse 962.

The hair in the mane and tail became loose at about the same time as in horse 962, and just before death the hair in both mane and tail was very loose. In addition, occasional periods of nervousness appeared as previously described and the same depravity of appetite was noted.

Post-mortem examination showed the carcass to be very thin. The blood was dark red in color. Aside from a slight emphysema and a few fibrinous tags on the visceral pleura, the lungs appeared more or less normal. The fat in the mediastinum was gelatinous. A few petechiae were present in the gelatinous fat along the coronary groove. Small subepicardial hemorrhages occurred in the region of the coronary vessels and larger ones on the right auricle. A few large hemorrhages were found on the right auriculoventricular valves. The heart muscle was pale and had a cooked appearance. The surface of the liver, which presented a few fibrinous tags, was yellowish in color and the lobules were hemorrhagic. On sectioning, the liver tissue was found to be of an ocher color and the lobules stood out distinctly. Numerous small hemorrhages occurred under the capsule of the spleen. There were also many ecchymotic hemorrhages in the omentum and mesentery. The cortex of the kidney contained some petechiae, the medulla was reddened, and the pelvis was filled with clear, gelatinous urine. The intestines showed some enteritis, and the peritoneum was reddened.

MULE 1034

Although mule 1034 received a slightly smaller quantity of selenium per day than did horses 962 and 980, it died much sooner, about 6½ months after the experiment began. The concentration of selenium

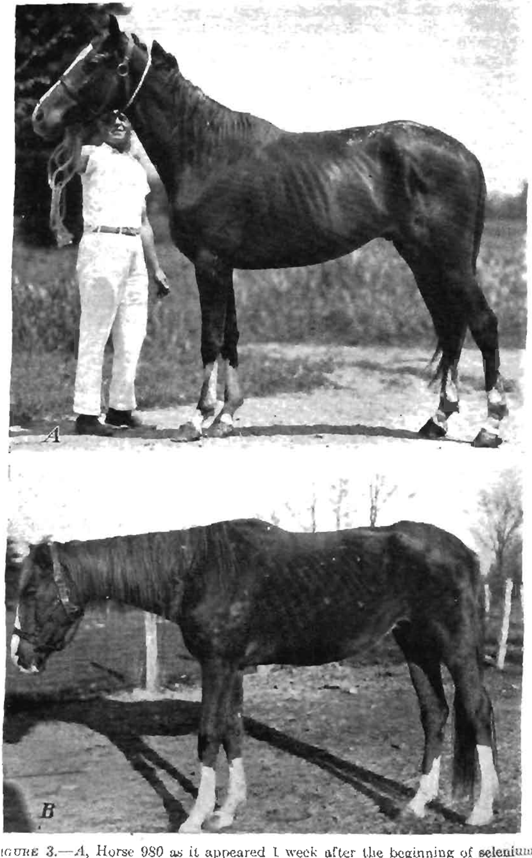


FIGURE 3.—A, Horse 980 as it appeared 1 week after the beginning of selenium feeding, and B, as it appeared 3 days before death occurred, or about 17 months after the beginning of the experiment. Note emaciation, drowsiness, and matted hair of mane in B.

was 17.3 parts per million of feed at the beginning of the experiment. In spite of the fact that the appetite was fairly good until a day or two before death, it will be seen in figure 1 that the animal's weight began to decrease within 2 weeks after selenium was added to the diet. The decline was gradual for about 2 months and then became much faster until death occurred. During the selenium-feeding period, there was a loss in weight of about 200 pounds.

Aside from emaciation, drowsiness, and some roughness of the horn at the top of the hoofs, relatively few symptoms were noted in this animal. Its condition 1 week after the experiment began is shown in figure 5, *A*; and *B* shows its appearance 4 months later, or 2½ months before death. At that time there had been a loss of about 100 pounds in weight. Changes in the horn at the top of the hoofs, manifested by a narrow white band particularly noticeable on the left front hoof, together with perceptible swelling of the coronary band, had just

FIGURE 4.—Front feet of horse 980 after 1 year from the beginning of the experiment. Note swelling of the coronary band, *a*, and the ring of roughened horn on the wall of the hoof below the hair line, *b*.

begun to appear. This was somewhat later than the initial appearance of such changes in the hoofs of the horses. Loosening of the hair, however, appeared in this animal at about the same time as in the horses, and at the time of death the hair in the mane was loose.

Post-mortem examination of the carcass showed it to be in very poor condition. The upper portion of each hoof had a slightly bulging appearance, and there was some roughening of the horny wall just below the coronary band. The pleural cavity contained about 300 to 400 cc. of clear, reddish yellow fluid. There were a few pneumonic areas in the posterior lobe of the left lung as well as in the anterior lobe of the right lung, which also showed some passive congestion. A small quantity of gelatinous fat was present around the pericardium and along the coronary groove of the heart. The pericardial sac was thickened, and a few petechiae were found on the epicardium. A

few small gray patches were found on the endocardium of the lef ventricle and some small subendocardial hemorrhages in the right ven-

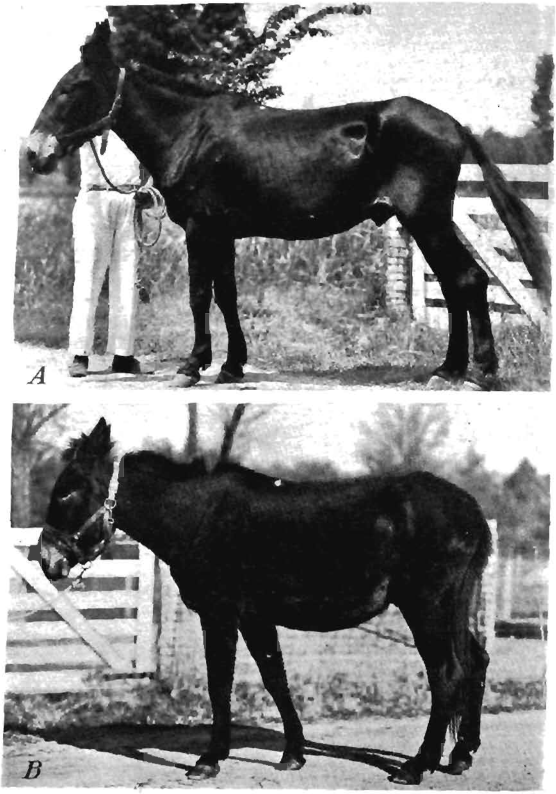


FIGURE 5.—*A*, Mule 1034 as it appeared 1 week after the beginning of selenium feeding, and *B*, after 4 months of selenium feeding and about 2½ months before death.

tricle as well as on the mitral valve. When sectioned, the myocardium was chocolate colored. Aside from being slightly darker in

olor, the liver appeared more or less normal. Nothing unusual was ound in the spleen or the intestines. There was some nephritis in he kidneys, and the bladder, which was distended with slightly loudy, yellowish urine, showed a mild catarrhal cystitis. The articuar surfaces of both the hip and stifle joints were slightly pitted and oughened, and each joint contained a small quantity of thick stringy uid.

HORSE 1023

Horse 1023 was included in the experiment to determine what ffect the high level of selenium (96 p. p. m.) which the other two orses were receiving would have on a horse that had not previously een given selenium. The animal died 35 days after being placed on he selenium diet. The changes in the weight are shown in figure 1. lthough the horse never ate all the feed given at any time and conseuently did not get the full dose of selenium, there is no doubt that he loss of weight was largely due to the action of the selenium. bout 4 days after the first dose was given, diarrhea appeared and ooseness of the bowels persisted until the animal's death. A few ours before death, the horse manifested colicky symptoms and eemed to be in a great deal of pain. Few symptoms of chronic elenium poisoning, other than emaciation, were observed in this horse.

Post-mortem examination showed the carcass to be very thin. There was slight scaling of the horn just below the coronary band of ll four hoofs. Some hypostatic congestion was found in the right ung, and there were subpleural hemorrhages on the right side. The at of the heart was yellow and gelatinous, and a few small hemorrhages ppeared in the fat along the coronary groove, as well as subepicardilly on the left ventricle. Massive subendocardial hemorrhages were resent in the left ventricle and petechiae in the right one. There as cirrhosis of the liver and the lobules stood out prominently. he spleen showed numerous small black petechiae under the capule. Hemorrhages occurred in the fat around the kidneys, the capules of which were removed with difficulty, and the kidney tissue self was very friable. The cortex contained pin-point hemorrhages, nd the medullary portion was reddened. In the mesentery, the fat issue was yellow and gelatinous and large hemorrhages were sprinled throughout. The stomach was found to be filled with oats, hereas the intestines were empty and slightly inflamed.

CHEMICAL DATA

The urine and feces were found to contain comparatively large uantities of selenium. These data are given in figures 6 and 7. he total quantity of excreta was not determined so that no estimation f the total quantity of selenium eliminated can be given. However, e high concentration in the excreta suggests that a large part of the ngested selenium was eliminated. From the analysis of portions of e horse carcasses and from analyses of small animals for selenium (7), is believed that the average concentration for horses would be less an 1 part per million. Horse 962 weighed 660 pounds at death. If is assumed that the carcass contained 1 p. p. m. of selenium, 0.3 m. of approximately 250 gm. ingested would have been retained in the ody of the animal.

There was an irregular but gradual increase in the selenium content of the blood while the horses were on the diet containing 24, and the mule 17.3, parts of selenium per million parts of feed (fig. 8). When the selenium content of the diet of the horses was increased to 48 p. p. m., the concentration in the blood became greater. The increase in the selenium content of the diet to 96 p. p. m. caused the concentration in the blood to continue to increase. At death the blood of horse 962 contained 9 p. p. m., and 3 days before death the blood of horse 980 contained 6 p. p. m. For both animals this was a sharp increase during the last week from a level of approximately 3.5 p. p. m. Horse 1023, which was begun on the high selenium diet, lived about 5 weeks and also showed a sharp increase in selenium concentration in the blood, ending with 9 p. p. m. at death. Mule 1034 died shortly after the

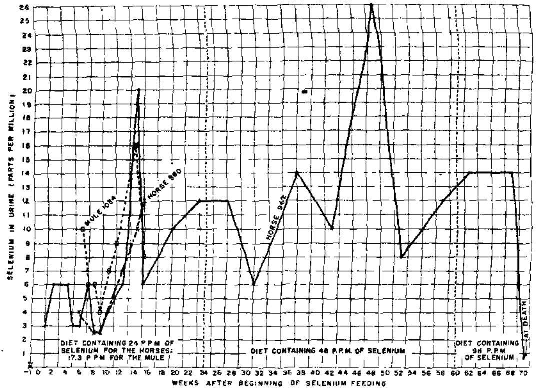
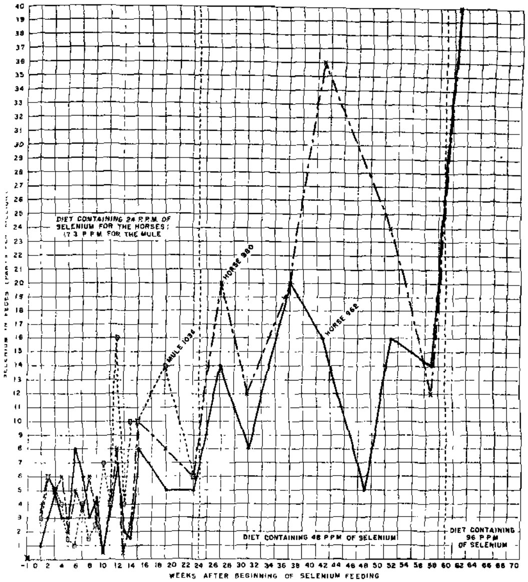


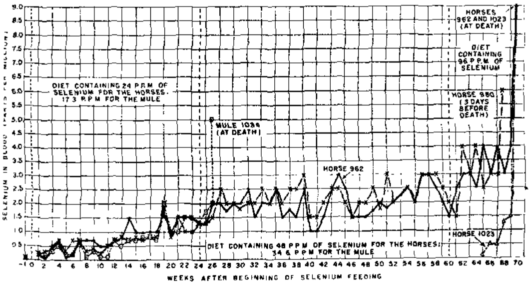
FIGURE 6.—Selenium content of urine from equines on a diet containing sodium selenite.

change from 17.3 to 34.6 p. p. m., and during the 3 days before death the selenium concentration of the blood changed from 2 to 5 p. p. m.

In table 1 are given the analyses of various parts of the body of the horses and mule that died as a result of small daily doses of sodium selenite, together with data obtained on horses and a mule that died from a single dose of sodium selenite. The concentration of selenium in the blood of the animals that died as a result of daily doses of selenium is appreciably higher than in that of the animals that died from a single dose. The kidneys of those that died from a single does were higher in selenium than the kidneys of those that received daily doses. This finding was reversed in the case of the spleen, as is shown by data from the horses that survived more than a year (Nos. 962 and 980). The hoofs of the animals that received daily doses had an accumulation



IGURE 7.—Selenium content of feces from equines on a diet containing sodium selenite.



'IGURE 8.—Selenium content of blood from equines on a diet containing sodium selenite.

of selenium. The concentration became greatest in the old hor portion of the hoof. In the other portions examined, no clear-c difference was found between the animals killed with a single d and those that died as a result of repeated small doses of selenium.

TABLE 1.—*Selenium content of material from equines that died from repeated sm doses of sodium selenite and from equines that died from a single dose*

Animal designation	Method of administering selenium	Selenium in—								
		Blood	Urine	Kidneys	Liver	Lungs	Spleen	Heart	Hair	H
		P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p*
Horse 1023	Daily doses of selenium for 5 weeks.	9.0	0.2	1.5	2.0	0.5	0.5	0.2	5.0	
Mule 1034	Daily doses of selenium for 26 weeks.	5.0	8.0	2.0	1.5	2.0	.7	.3	------	(1
Horse 980	Daily doses of selenium for 68 weeks.	[2] 6.0	------	5.0	2.5	2.5	3.5	.2	------	(3
Horse 962	Daily doses of selenium for 70 weeks.	9.0	.7	3.5	5.0	2.0	5.0	1.0	2.5	(4
Horse 764 [5]	Single lethal dose of 4.6 mg. per pound of body weight.	2.0	------	10.0	5.0	1.5	.4	.4	------	
Horse 875 [5]	Single lethal dose of 3.65 mg. per pound of body weight.	2.5	------	8.0	8.0	1.5	.5	.4	------	
Horse 1009 [5]	Single lethal dose of 2.0 mg. per pound of body weight.	------	1.4	10.0	2.0	.7	.5	.5	------	----
Mule 1035 [5]	Single lethal dose of 1.46 mg. per pound of body weight.	2.0	4.0	6.0	2.0	1.0	1.0	1.0	------	----

[1] Top one-third, 0.5 p. p. m.; middle one-third, 1.2 p. p. m.; bottom one-third, 1.2 p. p. m.
[2] Concentration 3 days before death.
[3] Inside soft material of top one-third, 0.3 p. p. m.; outside horny material of top one-third, 1.0 p. p. r inside soft material of middle one-third, 0.5 p. p. m.; outside horny material of middle one-third, 5.0 p. p. inside soft material of bottom one-third, 5.0 p. p. m.; outside horny material of bottom one-third, 8.0 p. p.
[4] Inside soft material of top one-third, 1.0 p. p. m.; outside horny material of top one-third, 5.0 p. p. r inside soft material of middle one-third, 1.0 p. p. m.; outside horny material of middle one-third, 2.0 p. p. r inside soft material of bottom one-third, 4.0 p. p. m.; outside horny material of bottom one-third, 10 p. p.
[5] By Miller and Williams (*4*).

DISCUSSION

From the results of this experiment, there appears to be little doul that chronic poisoning can be induced in equines when repeated sma doses of selenium are fed in the form of sodium selenite. Under tl conditions of this experiment, however, all the characteristic sympton of chronic selenium poisoning which have been described in anima maintained on naturally selenized feed (*2*, *5*) have not been repr duced, even during a prolonged period of feeding. Emaciation a drowsiness were marked in all four of the animals. In the two hors fed for the longest period, slight changes occurred in the character the horn of the hoofs. In addition, the long hair of the mane an tail became loose, but extreme depilation or these parts or sloughin of the outer wall of the hoof was never observed. On the other han the lesions present at autopsy, namely, the changes in the heart, live and spleen, were similar to those reported as occurring in natur cases of the disease (*1*, *5*).

In this connection, it is of interest to contrast the effects, in equine and swine, of continued feeding of small doses of selenium as sodiu selenite. In the latter species 50 percent of the animals exposed t selenium as sodium selenite manifested typical clinical sympton

ithin 3 months (*3*), whereas in equines certain characteristic symp-
ms of chronic selenium poisoning failed to develop in 17 months.
ome explanation for this difference may be found in the fact that the
vine ate relatively little selenized feed after the first few days of the
xperiment, whereas no impairment of appetite was noted in the
orses until immediately before death. This fact might account,
ierefore, for the increased resistance of the equines to the action of
ie selenium. When single large doses of selenium were given to
quines and swine, however, the results were reversed (*4*). In that
xperiment it was found that the minimum lethal dose for horses was
bout 1.5 mg. per pound of body weight, whereas 6 mg. per pound of
ody weight only caused the swine to become sick.

In view of the results found in the present experiment and those
ivolving single doses of selenium, it is believed that some tolerance
) the action of selenium, at least in the inorganic form, may be
equired by some animals. The number of equines included in the
resent experiment is too small to permit a definite conclusion to be
rawn. It should be pointed out, however, that about 6 months
fter selenium feeding began, horse 962 received daily 0.58 mg. of
elenium per pound of body weight, horse 980 received 0.53 mg., and
iule 1034 received 0.48 mg. The mule lived only 3 weeks at this level
f feeding, but the two horses showed relatively little ill effect.
bout 15 months after the experiment began, the quantity was again
icreased, and the relation of selenium to body weight for horse 962
as 1.20 mg. daily per pound and for horse 980, 1.12 mg. Since
ie animals were losing weight regularly the quantity of selenium
ontinued to increase in relation to body weight. When it is con-
dered that a single toxic dose of the element has been found to be
5 mg. per pound of body weight for horses and that one horse
No. 980) survived for nearly 2 months and the other one (No. 962)
ved 2½ months on daily doses almost as large, it would seem that
iese animals had built up some tolerance for selenium. This
elief is further strengthened by the result of feeding about the
ime concentration of selenium to horse 1023. When this animal
as placed on experiment, it received 1.19 mg. of selenium per day
er pound of body weight and it survived but 1 month.

During the approximately 2 months that horses 962 and 980 lived
fter the selenium content of the diet was increased to 96 p. p. m.,
iere was a gradual increase in the selenium content of the blood,
iarked by a sharp increase just prior to death. During the last
) days the appetite decreased until the intake of selenium was
elatively small. These facts suggest that there was a break-down
i the elimination of selenium from the blood. The urine sample
iken from horse 962 at death contained but 0.7 p. p. m. as compared
ith 6 to 26 p. p. m. during the whole previous year.

SUMMARY

To study the effect on equines of long-continued feeding of selenium
inorganic form, small daily doses of sodium selenite were adminis-
ered in the feed to three horses and a mule. The animals were
pparently in good health when the experiment began. The work

was carried on in 1937 and 1938 at the Animal Disease Stati Beltsville, Md.

At the beginning of the experiment a measured quantity of M/ solution of sodium selenite was added to the oats at the rate of parts of selenium per million of the total ration for two horses a 17.3 parts per million for the mule. The animals ate the seleniz oats readily at first, but after about 2 months refused them and ate on hay. The sodium selenite solution was then given as a drench for abo 4 months, when the quantity of selenium was doubled, making a lev of 48 p. p. m. for the horses and 34.6 for the mule. About 4 montl later, because of difficulty in drenching the horses, the mule havin died in the meantime, the method of administering the sodium selenit was changed so that the solution was diluted and sprinkled on both th oats and hay. The level of selenium was raised to 96 p. p. m. of fee for the two horses about 15 months after the beginning of the experi ment. A month later a third horse was placed on experiment an immediately given approximately 115 parts of selenium per millio parts of feed daily.

The mule survived for only 6½ months after selenium feeding began, but one of the horses lived about 17 months and another 17½ months. The third horse, which received the highest level of selenium at the beginning of the experimental feeding, lived only about 5 weeks. The principal symptoms of chronic selenium poisoning exhibited by these animals were emaciation in spite of a fairly good appetite, listlessness, looseness of the hair of the mane and tail, and softening and scaling of the horny wall of the hoof. None of these symptoms, however, were so outstanding as those described in cases of chronic poisoning which occur under natural conditions. The lesions observed in the heart, liver, spleen, and kidneys at autopsy resembled those described in field cases, to a large extent.

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FEATHERING, GROWTH, FEED CONSUMPTION, AND RACHITOGENESIS IN CHICKS AS INFLUENCED BY THE KIND OF GRAIN IN THE DIET [1]

By H. L. Wilcke, *collaborator*, and John C. Hammond, *associate biologist, Animal Nutrition Division, Bureau of Animal Industry, United States Department of Agriculture* [2]

INTRODUCTION

The cereal grains occupy a prominent position in the diets of poultry of all ages, yet definite information regarding the value of each of these cereals for specific purposes is lacking. Only within the last few years have there been active attempts to compare the cereal grains used with respect to their growth-promoting and bone-forming properties for chicks. The feathering of growing chicks is important to the broiler producer and to all producers of chicks because well-feathered broilers sell to much better advantage than others and because there is less danger of cannibalism among well-feathered chicks.

REVIEW OF LITERATURE

Since the literature has been reviewed in a recent article by Branion, Stackhouse, and Hull (*1*),[3] it is necessary only to add a few comments on data which have appeared since their paper was published. Branion and his coworkers (*1*) found no evidence of any anticalcifying property of oats or oat groats in comparison with corn, wheat, or barley.

The Council on Foods of the American Medical Association (*3*) published a report in which it was concluded that the experimental results available "may be explained on the basis of the calcium and phosphorus ratio in the diet together with a knowledge of the availability of the phosphorus," and further, "that there is not good evidence for the existence of a decalcifying factor in cereals." These conclusions are further substantiated by Mottram and Palmer (*9*), who found that the rickets produced by cereal diets was due to a low calcium content of the diets and that this condition could be corrected by the addition of calcium or of vitamin D. Rickets was not prevented by the addition of available phosphorus. These results do not agree with the explanation offered by Bruce and Callow (*2*), who suggested that the rachitic condition was caused by a lack of available phosphorus in the diets.

McDougall (*7*) found that the addition of 11 percent of lard or olive oil to the diet prevented rickets in rats which were fed diets low in calcium and vitamin D and made up largely of wheat flour or bread.

[1] Received for publication April 12, 1940.

[2] By authorization of the Bureau of Animal Industry, H. L. Wilcke, research professor of poultry husbandry, Iowa Agricultural Experiment Station, collaborated in conducting this work at the U. S. Department of Agriculture, Beltsville Research Center, Beltsville, Md. The authors are indebted to M. A. Jull, formerly of the U. S. Department of Agriculture, for arranging for the collaboration; to Harry W. Titus for his assistance, advice, and helpful criticisms; and to C. A. Denton and H. E. McClure for analyses of feed and bones.

[3] Italic numbers in parentheses refer to Literature Cited, p. 379.

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The action of the fat or oil is based on the increased absorption c calcium due to the formation of calcium soaps.

Miller and Bearse (*8*) presented evidence to show that pullets fe a growing and laying ration with oats as the sole cereal developed les cannibalism than those fed a ration containing corn as the sole cereal In addition, Kennard and Chamberlin (*6*) have published result indicating that oats have a definite value in the laying ration. Th review by Crampton (*4*) of work comparing the various cereal grain for both chicks and laying birds demonstrates clearly the need fo additional information regarding the relative value of the cerea grains in avian nutrition.

PURPOSE AND PLAN OF THE EXPERIMENT

The purpose of this experiment was to study the effect of oats barley, corn, and wheat on feathering and growth, on the percentag of ash in the tibiae and their size, and on the feed consumption o chicks receiving diets with each of these grains as the principal cerea portion. Particular emphasis was placed on oats, since some of th earlier work had indicated a rachitogenic factor in this cereal whe rats or puppies were the experimental animals. The experiment wa conducted between May 15 and August 7, 1936, at the United State Department of Agriculture, Beltsville Research Center, Beltsville, Md

Six hundred Single-Comb White Leghorn chicks were selected fron a larger group which had been hatched from eggs from mass-mate pullets at the Beltsville Research Center. These chicks were hatche on May 15, 1936, and the following morning they were leg-banded b the use of duplicate bands. The chicks were weighed to one-tenth of gram on an automatic scale, the weight of the bands being balance with two bands on the scale. The chicks were then divided into 1 lots of 50 each, placed at random in the various lots. Each lot wa kept in a separate colony brooder house in which sand was used as litter, and the chicks were fed and watered immediately. None o them were allowed out of the houses at any time during the experiment The lots were numbered from 412 to 423 to correspond to the die numbers, and the chicks were weighed at biweekly intervals.

The plan of the experiment was to feed four lots of chicks all-mash diets made up of a single cereal grain (oats, barley, corn, or wheat) with dried buttermilk as the protein supplement, and sufficient high-grade ground limestone and chemically pure tricalcium phosphate to adjust the calcium and phosphorus to equal levels.

In order to determine the fraction of the grain in which the rachitogenic factor might be found, if it proved to be present, lots were included in which 20 percent of ground oat hulls supplemented corn and wheat. It was calculated that the oats diet contained approximately 20 percent of hulls, and this level was used to equalize the quantity of hulls in these diets with that in the oats diet. In addition, lots were included in which 20 percent of ground hulled oats supplemented the corn and the wheat, and also lots in which 20 percent of ground whole oats supplemented the corn, wheat, and barley. Finally, one lot on a standard mixed diet was included in the experiment as a control lot.

All the diets except the control were made up to contain as nearly as possible 16.6 percent of protein (dried buttermilk was used as the only

rotein supplement in order to keep this part of the diet uniform), .75 percent of calcium, and 0.54 percent of phosphorus. In addition, percent each of salt (sodium chloride) and cod-liver oil was used.

TABLE 1.—*Composition of the experimental diets except the control*

Ingredient	Parts, by weight, of diet No.—										
	412	413	414	415	416	417	418	419	420	421	422
iround whole oats	77								20	20	20
iround yellow corn			66¾				42½	52	49¼		
iround barley		74									54½
iround wheat				77	49¼	59⅞				57	
iround hulled oats						20		20			
iround oat hulls					20		20				
ried buttermilk	21	24	31½	21	29	18	36	26	29	21	23½
iround limestone	1	1	½	1	⅝	1	¼	¾	⅛	1	1
alcium phosphate			¼		⅛	⅛	¼	¼	⅝		
alt	1	1	1	1	1	1	1	1	1	1	1
od-liver oil	1	1	1	1	1	1	1	1	1	1	1
Total	101	101	101	101	101	101	101	101	101	101	101

It was not possible to analyze the ingredients before the diets were nade up, but samples of each ingredient were taken as the diets vere mixed, and analyses were obtained later. One hundred pounds f each diet was mixed without cod-liver oil. The oil was incorporated o the feed at least once every 2 weeks. Only as much feed was mixed s would be used during that period. Table 1 shows the composition f all the diets except the control diet. The composition of the control liet (No. 423) was as follows:

	Parts by weight
Ground yellow corn	25
Wheat middlings	25
Rolled oats	10
Dried skim milk	10
Corn gluten meal	10
Alfalfa-leaf meal	8
Wheat bran	5
Yeast cells	3
Ground limestone	1. 5
Salt	1. 0
Cod-liver oil	1. 5
Total	100

When the chicks were 4 weeks of age, they were classified as to eather development, those which were completely feathered on the lorsal region being scored as group 1, those partly feathered as group , and those with only down on the backs as group 3.

Growth was measured by increase in weight at biweekly intervals or 12 weeks and also by three measurements made on the tibiae of ive representative males and five representative females at 8 and 12 veeks of age. These measurements were (1) the length of shaft, aken with the epiphyseal cartilages removed, (2) the minimum diamter of the shaft at its midpoint, and (3) the maximum transverse liameter of the proximal head. After the bone measurements had een taken on the tibiae from the 8-week-old chicks, these bones were xtracted and ashed according to the method described by Harshaw, ritz, and Titus (*5*).

The feed was weighed for each lot at biweekly intervals, feed weig being taken on the same day as the chick weights. Feed consump per chick per day and the total quantity consumed per chick for entire period were calculated.

EXPERIMENTAL RESULTS

FEATHERING

The results of the classification of the chicks into feather gro and the number of males and females in each of these groups presented in table 2.

TABLE 2.—*Number of chicks at 4 weeks of age in each of three feather group affected by the grain in their diet*

Lot No.	Kind of grain in diet	4-week-old chicks in—			
		Group 1	Group 2	Group 3	All gr
		Number	*Number*	*Number*	*Num*
412	Oats	45	3	1	
413	Barley	36	13	1	
414	Corn	1	10	37	
415	Wheat	7	27	15	
416	Wheat and oat hulls	4	31	15	
417	Wheat and hulled oats	0	25	25	
418	Corn and oat hulls	0	23	27	
419	Corn and hulled oats	8	24	15	
420	Corn and oats	0	13	34	
421	Wheat and oats	9	28	12	
422	Barley and oats	26	21	2	
423	Mixed (control)	2	23	25	
Total		138	241	209	
Males		61	125	108	
Females		77	116	101	

It is obvious that the chicks in the various lots differed in resp to feather development. The question arose immediately as whether the better feathering was due to the diet or whether it v associated with superior general development. In order to vestigate this point, the chicks were grouped by sex into the th feather groups regardless of the diet which they had been fed. Tl weights were analyzed by means of analysis of variance, the meth of Snedecor (*10*) being used. Differences due to a disproportion distribution of the sexes within the 12 lots were thereby eliminat

A highly significant difference was found between the weights the males and females. It is rather unusual to find a pronoun difference in the weights of the sexes at only 4 weeks of age; nevertl less, such differences were found for all the chicks in this experime

There were also highly significant differences among the weig for the three feather groups. Within each sex, the chicks wh feathered most rapidly and, consequently, were classed as group 1 feathering, were also the largest chicks. The data indicate tl those diets which promote most rapid growth produce chicks that a most completely feathered at 4 weeks of age. This result is probat associated with rapid general development rather than with the di However, there was a distinct difference in the quality of the feath ing of the various groups. The oat-fed chicks had a much super quality of feather, whereas the corn-fed chicks were rated lowest

this respect. The feather structure appeared to be modified slightly by the diet. The corn-fed chicks were rough in appearance, and many of them developed feathers which curled forward, presenting a frizzled appearance. The barbs, barbules, and barbicels all seemed to be present, but the barbicels did not appear to function normally in that the web of the feather was not smooth. This feathering is in marked contrast to that of the oat-fed group, which developed much smoother feathers. The addition of whole oats, oat hulls, or hulled oats improved the feathering of the chicks fed corn. This finding is direct evidence that there is some substance, factor, or group of substances or factors in oats which exerts a beneficial effect on growth and on the quality of feathering, but it was not possible to determine them from the results of this experiment.

The relationship between sex and feathering is not so pronounced. The interaction between these two characters was significant, but not

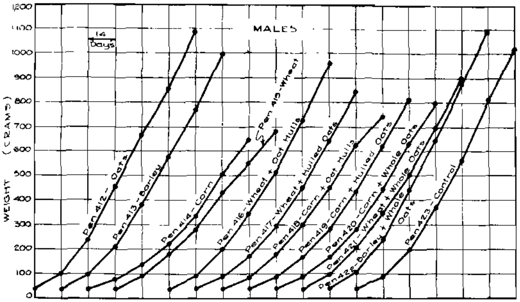

FIGURE 1.—Mean weights of males at biweekly intervals for 12 weeks.

highly so, indicating that there is probably a slight advantage in favor of the females in rate of feathering.

GROWTH AS MEASURED BY LIVE WEIGHT

The weights of the males and the females in the 12 lots are presented in figures 1 and 2. Since bone-ash determinations and bone measurements were made at 8 and at 12 weeks, the weights were also analyzed for differences at these periods, and, in addition, at the 10-week period, in order that these results might be compared with previous results in which analyses of data had been made only for the weight at 10 weeks.

The results of the statistical analysis were essentially the same for the three periods selected. There were highly significant differences in the weights of the chicks in the different lots at 8, 10, and 12 weeks. These results *indicate that* the diets affected the rate of growth of these chicks. However, in order to compare the individual diets, a more detailed analysis was necessary, and for this purpose the method suggested by Snedecor (*10*) was used to find the least mean difference which would be significant or highly significant.

The least differences which would be significant for the 8-, 10-, an 12-week data, respectively, were 35.67, 54.46, and 64.09 gm., and th least differences which would be highly significant were 46.96, 71.6! and 84.37 gm. Since the number of birds per lot varied only fro 47 to 50, an arbitrary value of 48 chicks per lot was used in calcu lating these significant values for the 8-week data, and since 1 chicks had been removed from each lot at the end of the eighth wee the number per lot used for the 10- and 12-week data was 38.

An examination of the weight means for both males and females a presented in table 3 reveals the following significant differences:

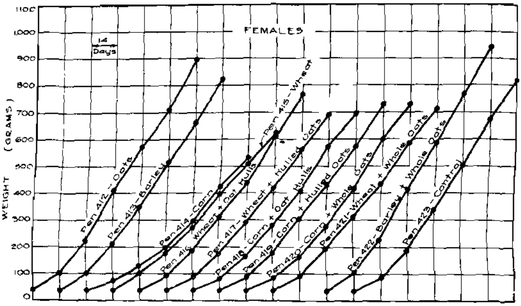

FIGURE 2.– Mean weights of females at biweekly intervals for 12 weeks.

TABLE 3.—*Average combined weights of male and female chicks in the 12 lots a affected by the grain in their diet*

Lot No.	Kind of grain in diet	Live weight at— 8 weeks	10 weeks	12 weeks	Lot No.	Kind of grain in diet	Live weight at— 8 weeks	10 weeks	12 weeks
		Grams	*Grams*	*Grams*			*Grams*	*Grams*	*Grams*
412	Oats	620	781	990	418	Corn and oat hulls	432	595	71
413	Barley	545	716	913	419	Corn and hulled oats	433	598	77
414	Corn	317	469	594	420	Corn and oats	429	612	75
415	Wheat	415	530	650	421	Wheat and oats	462	630	78
416	Wheat and oat hulls	484	682	883	422	*Barley and oats*	*620*	*830*	*1,03*
417	Wheat and hulled oats	435	597	760	423	Mixed (control)	634	744	92

The corn-fed chicks, lot 414, were definitely smaller than those in any other group.

The wheat-fed chicks, lot 415, were heavier than the corn-fed chicks but weighed less than those in any other group.

The chicks fed oats and those fed barley and oats, lots 412 and 422, were definitely larger than those in any of the other groups.

The addition of ground oat hulls to wheat increased the weight of the chicks more than did the addition of an equal quantity of either ground hulled oats or ground oats.

The addition of 20 percent of ground oat hulls, ground hulled oats, or ground oats to corn increased the weight of the chicks over that of those fed corn as the sole grain, and the differences in weight of the chicks fed these three supplements were not significant.

The differences in weight ranked in essentially the same order at 8, 10, and 12 weeks.

These results do not appear to be in complete agreement with those of Branion and his coworkers (*1*). However, the diets were not entirely comparable, since in the present work there were no more than two cereals in any one diet and only one source of protein was used.

There was a highly significant difference in the weights of the males and females at 8, 10, and 12 weeks, but this was to be expected. The interaction between sex and diets was not significant, indicating that the males and females reacted similarly to the diets fed.

GROWTH AS DETERMINED BY MEASUREMENTS OF TIBIAE

The mean measurements of the tibiae of the males and females at 8 weeks and at 12 weeks are presented in table 4.

TABLE 4.—*Mean measurements of the tibiae of the male and female chicks in the various lots at 8 and 12 weeks of age as affected by the grain in their diet*

MEASUREMENTS FOR MALES

Lot No.	Kind of grain in diet	At 8 weeks of age			At 12 weeks of age		
		Length of shaft	Diameter of shaft	Diameter of proximal head	Length of shaft	Diameter of shaft	Diameter of proximal head
		Cm.	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
412	Oats	3.28	0.165	0.648	4.13	0.203	0.811
413	Barley	2.89	.160	.633	4.09	.196	.766
414	Corn	2.01	.127	.498	3.85	.184	.740
415	Wheat	2.95	.150	.562	3.77	.187	.708
416	Wheat and oat hulls	3.00	.144	.567	4.02	.195	.781
417	Wheat and hulled oats	2.95	.149	.546	3.80	.192	.741
418	Corn and oat hulls	2.93	.143	.562	3.86	.191	.754
419	Corn and hulled oats	2.88	.143	.549	3.95	.203	.772
420	Corn and oats	2.91	.143	.574	3.96	.195	.754
421	Wheat and oats	3.02	.147	.564	3.89	.184	.774
422	Barley and oats	3.30	.168	.628	4.26	.210	.828
423	Mixed (control)	2.95	.147	.517	4.20	.201	.806

MEASUREMENTS FOR FEMALES

Lot No.	Kind of grain in diet	At 8 weeks: Length of shaft	At 8 weeks: Diameter of shaft	At 8 weeks: Diameter of proximal head	At 12 weeks: Length of shaft	At 12 weeks: Diameter of shaft	At 12 weeks: Diameter of proximal head
412	Oats	3.10	0.159	0.592	4.06	0.206	0.728
413	Barley	3.29	.157	.574	4.01	.201	.698
414	Corn	2.43	.131	.485	3.61	.186	.644
415	Wheat	2.87	.152	.531	3.84	.190	.676
416	Wheat and oat hulls	2.80	.139	.514	3.93	.201	.713
417	Wheat and hulled oats	2.87	.140	.552	3.72	.187	.668
418	Corn and oat hulls	2.70	.138	.571	3.72	.186	.674
419	Corn and hulled oats	3.03	.142	.551	3.81	.191	.682
420	Corn and oats	2.75	.137	.520	3.91	.195	.718
421	Wheat and oats	2.74	.136	.524	3.72	.176	.647
422	Barley and oats	3.19	.168	.594	4.16	.202	.723
423	Mixed (control)	3.11	.145	.576	4.07	.202	.701

When these data were analyzed by the method of Yates (*11*) to adjust for missing data, it was found that the differences between lots were significant for all three measurements—length of shaft, diameter of shaft, and diameter of proximal head—at both 8 and 12 weeks of age. In addition, there were highly significant differences

between the sexes in length of shaft and diameter of the proxima head of the tibiae but not in diameter of the shaft. In general, th males had the longer bones and also the larger diameter of the proxima head, as would be expected because of their greater size and weight but this relationship did not hold for the diameter of the shafts o these bones. The interaction between sex and diet was not significant indicating that the sexes responded similarly to the various diets.

These results indicate that undoubtedly there are differences in the bone measurements for the various lots of chicks, but her again the question arises as to whether these differences are due t the diet or to general development. In order to clarify this poin somewhat, the method of analysis which was used in connection witl the weights was applied to these data. The least mean difference found to be significant and highly significant were as follows:

(1) For the diameter of the proximal head, the least difference that are significant and highly significant are approximately the sam for both the males and the females at both 8 and 12 weeks, namely 0.054 and 0.081 cm., respectively. (2) For the length of the shaft the least differences that are significant and highly significant ar 0.33 and 0.50 cm., respectively, for the males at 8 weeks, 0.42 an 0.61 cm., respectively, for the females at 8 weeks, and 0.71 and 1.0 cm., respectively, for both the males and the females at 12 weeks (3) For the diameter of the shaft, the least differences that are signi ficant and highly significant are 0.018 and 0.027 cm., repectively, fo the males at 8 weeks, 0.005 and 0.007 cm., respectively, for the male and the females at 8 weeks, and 0.006 and 0.009 cm., respectively for both the males and the females at 12 weeks. In making thes calculations the same values were used for both sexes only when th variance was practically the same.

When the means of the various lots were compared, the above mentioned measures for significant and highly significant difference being used, it was found that at 8 weeks the males on the corn die had much shorter tibiae than those on several of the other diets Significant differences also existed between other lots of males an between three lots of females. These results seemed open to question and the data were reanalyzed by the use of the cube root of liv weight instead of live weight itself. When the effect of the cub root of live weight was removed in this manner, no significant dif ferences were found between the means for length of tibiae of eithe males or females at 8 or 12 weeks of age. Likewise, with the sam procedure no significant differences were found between the mean for diameter of proximal heads or diameter of the shaft of the tibia in either sex at 8 or 12 weeks of age.

BONE ASH

The mean percentage of bone ash of the tibiae, with epiphysea cartilages removed, for the males and the females are tabulated ir table 5. These data were analyzed by analysis of variance, the metho of Yates (*11*) being used to adjust for missing data.

The differences in percentage of bone ash of the males and female were not significant. This result is in agreement with those publishe by Harshaw, Fritz, and Titus (*5*), whose method of removing th epiphyseal cartilages was followed, and by whom it was pointe

.t that sex differences appeared only when the percentage of ash as determined with bones on which the epiphyseal cartliages had en retained.

\BLE 5.—*Percentage of bone ash in tibiae of male and female chicks in the various lots at 8 weeks of age as affected by the grain in their diet*

.ot No.	Kind of grain in diet	Bone ash in tibiae of— Males	Females	Both sexes	Lot No.	Kind of grain in diet	Bone ash in tibiae of— Males	Females	Both sexes
		Percent	*Percent*	*Percent*			*Percent*	*Percent*	*Percent*
12	Oats	55.14	55.96	55.55	418	Corn and oat hulls	57.73	57.41	57.57
13	Barley	55.83	57.61	56.72	419	Corn and hulled oats	57.95	57.69	57.82
14	Corn	56.62	57.23	56.93	420	Corn and oats	57.45	57.98	57.71
15	Wheat	57.57	57.19	57.38	421	Wheat and oats	56.61	56.65	56.63
16	Wheat and oat hulls	56.92	56.65	56.79	422	Barley and oats	56.91	56.16	56.53
17	Wheat and hulled oats	58.70	58.78	58.74	423	Mixed (control)	57.65	56.85	57.25

There were highly significant differences in the bone ash among the arious lots. When the individual lots were compared by combining ie figures for males and females, it was found that the bone ash of ie chicks fed the oats diet was significantly lower than that of all her lots. All the lots fed corn were definitely higher in bone ash ian those fed oats. The differences among the remaining lots were ot highly significant, except the lot fed wheat and hulled oats, which as the highest of any of the lots. The high ash content of the tibiae the chicks in this lot can be accounted for only on the basis of the illed oats, since lot 419, fed corn and hulled oats, the only other et in which hulled oats was used, is not significantly lower, whereas ts 415, 416, and 421, in which wheat was fed, are all highly sigficantly lower. However, since only two lots were fed hulled oats id, furthermore, since this feed constituted only 20 percent of the et of each, no definite conclusions can be drawn. The two lots fed .ets in which barley appeared were among the lowest in percentage bone ash, being significantly lower than six of the other lots. owever, the biological importance of the low bone ash in the barleyd lots may be questionable, since all values were well above the vel usually considered as normal.

FEED CONSUMPTION

The data on feed consumption are presented in table 6. It is obvis that there were differences in the quantity of feed consumed per ick per day in the various lots, but as has been shown, there were so differences in the growth of the chicks, and these differences were the same direction. That is, those lots of chicks which made the eatest gains in weight also consumed the most feed. Consequently, iese data were subjected to analysis of covariance by the method of iedecor (*10*). This analysis indicated highly significant differences nong the various lots in feed consumption. However, the differences nong the adjusted means for feed consumption were not significant, dicating that the various lots utilized the feed almost equally efficntly. Consequently, the differences in weight were a direct result increase in feed consumption rather than in efficiency of the feed nsumed.

TABLE 6.—*Feed consumption per chick in the various lots fed different grains*

Lot No.	Kind of grain in diet	Feed consumption per chick in 2-week period ending—						Total feed consumption per chick
		May 29	June 12	June 26	July 10	July 24	Aug. 7	
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
412	Oats	214.2	350.0	707.0	894.6	999.6	1,041.6	4,207.[illegible]
413	Barley	208.6	284.2	607.6	711.2	819.0	1,038.8	3,869.4
414	Corn	197.4	166.6	338.8	574.0	855.4	901.6	3,033.[illegible]
415	Wheat	207.2	214.2	455.0	574.0	856.8	884.8	3,192.[illegible]
416	Wheat and oat hulls	203.0	312.2	558.6	691.6	961.8	1,019.2	3,746.4
417	Wheat and hulled oats	214.2	203.2	362.6	543.2	924.0	882.0	3,129.[illegible]
418	Corn and oat hulls	198.8	229.6	499.8	702.8	845.6	942.2	3,418.[illegible]
419	Corn and hulled oats	176.4	197.4	385.0	631.4	859.6	1,006.6	3,256.4
420	Corn and oats	189.0	198.8	438.2	590.8	849.8	918.4	3,185.[illegible]
421	Wheat and oats	205.8	240.8	442.4	574.0	848.4	876.4	3,187.[illegible]
422	Barley and oats	214.2	326.2	572.6	813.4	1,003.8	1,008.0	3,938.2
423	Mixed (control)	190.4	252.0	483.0	729.4	953.4	950.6	3,558.[illegible]

DISCUSSION

It is a common opinion among poultrymen in general that corn is one of the most palatable grains used in poultry feeding. Nevertheless, in this work, the feed consumption of the chicks in the corn-fed lot was low throughout and the rate of growth of these chicks was correspondingly slow. The oats, barley, and oats and barley combination were utilized just as efficiently as the corn, and the addition to the corn of whole oats or of either the oat hulls or the hulled oats resulted in improvement of the corn diet. This was not expected in the case of the oat hulls, because there is a common supposition that an increase in the fiber content of a poultry diet, and particularly of a chick diet, will reduce the efficiency of that diet.

The only cases of cannibalism observed in this experiment were in the corn-fed lot of chicks. This finding is in agreement with results published by Miller and Bearse (*8*), who found that oats tend to prevent cannibalism in pullets.

At 4, 6, and 8 weeks of age, the chicks fed oats and those fed barley and oats were almost twice as large as those fed corn even though the former diets were fed at approximately the same level of protein, calcium, and phosphorus, and with a smaller supply of vitamins A and G complex than the corn diet.

The lower percentage of bone ash in the lots fed oats and barley than in the lot fed corn might be interpreted as indicating the presence of a rachitogenic factor in those two grains. However, the small reduction in bone ash caused by oats or barley at the high levels fed in these experiments would hardly be interpreted as of great biological importance, particularly when all ash values were at relatively high levels. In fact, it may be argued that it is equally undesirable to develop a bone ash that is excessively high, and in this case there is some question as to whether the oats and barley might carry a rachitogenic factor or whether corn may carry a factor for excessive calcification. In the results obtained here, neither hypothesis would be of biological significance because of the small differences obtained, but the results are interpreted as supporting the contention of Branion, Stackhouse, and Hull (*1*) that these cereals do not possess dissimilar properties in relation to calcification.

SUMMARY AND CONCLUSIONS

Twelve lots of Single-Comb White Leghorn chicks, each lot containing 50 birds, were fed oats, barley, corn, or wheat as the principal cereal portion of the diet. This work was carried on in 1936 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

Although the diet containing corn as the sole grain had larger quantities of vitamins A and the G complex than the other diets, the one with oats as the sole grain produced the most rapid gains of any of the diets containing single grains. The remaining single-grain diets ranked in the following order: Barley, wheat, and corn.

There appeared to be little difference in the efficiency of the diets used as measured by the units of feed necessary to produce a unit of gain in weight.

Chicks fed a diet in which corn was the sole grain had a very poor quality of feathering.

The addition of 20 percent of ground oat hulls, ground hulled oats, or ground whole oats to corn or to wheat diets improved the rate of growth and also improved the quality of feathering in the corn-fed chicks.

Oat hulls proved to be more effective than hulled oats or whole oats in supplementing wheat, but there seemed to be no difference in the value of these three products as supplements for corn.

The difference in bone development in the several lots was regarded as due to the effect of the diet on general development rather than to a specific effect on bone development.

Apparently oats and barley exert a slightly depressing effect on the percentage of ash in the tibiae of chicks, but this decrease in percentage of ash is slight and probably of little biological significance since the values for all lots were well above the minimum accepted as normal.

The data presented indicate that the four grains used do not possess rachitogenic properties.

Differences in rate of growth were due directly to differences in feed intake and not to differences in efficiency of the diets.

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THE EFFECT OF COOKING AND STORAGE ON THE ASCORBIC ACID CONTENT OF POTATOES[1]

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INTRODUCTION

Potatoes are consumed throughout a period beginning before their maturity and extending from 5 to 10 months after the tubers are harvested. During this period they undergo metabolic changes, the character and extent of which depend upon the duration and conditions of storage as well as upon the variety of potato. These changes are accompanied by changes in the ascorbic acid content. Since potatoes are never or rarely eaten raw, the effects of the numerous methods of cooking must also be considered if a true picture of their antiscorbutic value is to be obtained.

Although chemical methods of analysis simplify the quantitative determination of ascorbic acid, the application of these methods to biological material frequently creates new difficulties in the interpretation of results. Furthermore, in the case of the potato, insufficient information with regard to one or more of the common variables, time and temperature of storage, variety of potato, and cooking method, adds greatly to the difficulty of making a satisfactory comparison between the results of different investigators. Although the ascorbic acid content of raw potatoes is reported to vary between the wide limits of 53 mg. per 100 gm. (*10*)[3] and 1.5 mg. per 100 gm. (*13*), the results of most investigations indicate that newly harvested potatoes are highest in vitamin C value and that this quantity diminishes very rapidly during the first part of storage and later more gradually (*16, 18, 22, 26*). Kröner and Steinhoff (*8*) noted this decrease followed by a rise during the latter part of storage, and Pett (*18*) reported that sprouting caused an increase followed by a rapid decrease.

Fixsen (*4*) has summarized the data that show the effects of cooking upon the ascorbic acid content of potatoes. No general agreement exists. The same method of preparation reported by some investigators as causing a loss in ascorbic acid value, has been reported by others as apparently causing a gain; and one investigator reports that the same method of cooking results in a loss of ascorbic acid in one variety and a gain in another. Richardson, Davis, and Mayfield (*20*) reported a gain of 28 to 75 percent in ascorbic acid as a result of baking potatoes, whereas Lyons and Fellers (*12*) using the same method of cooking noted a loss of 50 percent. The gain in ascorbic acid found after cooking has been ascribed variously to the presence in the raw tuber of a protein-ascorbic acid ester capable of hydrolysis on cooking

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[3] Italic numbers in parentheses refer to Literature Cited, p. 393.

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(*9, 13, 16, 19,* 23), to the destruction of the ascorbic acid oxidase by heat (*3, 14*), and to the increased permeability of cooked tissue to the extracting medium, which results in greater ease of extraction of the vitamin from cooked than from raw potatoes (*16, 25*). It is hoped that the present investigation may point to definite conclusions on some of these controversial issues.

MATERIALS AND METHODS

SOURCE AND TREATMENT OF EXPERIMENTAL POTATOES

The Irish Cobbler and Green Mountain potatoes used in the major part of this study were furnished by the Bureau of Plant Industry and were grown at Aroostook Farm, Presque Isle, Maine. They were received on October 24, 1938, and placed in storage at 15.5° C. with a relative humidity of 80 percent. Six weeks later, half of the potatoes of each variety were removed to another storage room kept at 4.5° and a relative humidity of 80 percent. Sprouting was arrested at the storage temperature of 4.5° but continued in the potatoes stored at 15.5°. After a total of 4 months of storage, all sprouts were removed from the latter group. Any sprouts present were always removed at the time a potato was analyzed for ascorbic acid content.

The Irish Cobbler potatoes stored at 15.5° C. were used in a study of the effect of pressure cooking, baking, boiling (pared and unpared), and steaming (unpared) upon the ascorbic acid content of potatoes. For comparison, raw potatoes were analyzed simultaneously with potatoes cooked by each of these methods. These analyses of raw tubers, performed at intervals during the storage period, gave a history of the effect of storage at 15.5° upon the ascorbic acid content of Irish Cobbler potatoes. In addition determinations were carried out at intervals on the raw Green Mountain potatoes stored at 15.5° and on both varieties stored at 4.5°.

Since it was also desired to determine the effect of the so-called "waterless" cooking method upon the vitamin C value of potatoes, and there were not enough of the Maine-grown potatoes left in good condition, this part of the experiment was carried out on "new" Green Mountain potatoes obtained on the local market. Analyses were made of boiled pared potatoes and of pared potatoes cooked by the waterless method. Comparison was also made with raw potatoes simultaneously analyzed.

Summer Chippewa potatoes grown by the Bureau of Plant Industry at the experimental farm at Beltsville, Md., were made available for a study of the effect of maturity on the ascorbic acid content of potatoes. The analyses were begun 82 days after planting when the skins were still "feathery" and were made on the maturing tubers at semiweekly intervals for 6 weeks, by which time the vines were dead. The potatoes were delivered to the laboratory on the same day they were harvested and analyses were completed within 2 days. A disease, fusarium wilt, which ultimately became apparent in over half of these potatoes, developed at about the middle of the experimental period. Only tubers which appeared to be unaffected were analyzed. The effect of storage at 25.5° C. was also investigated.

SAMPLING PROCEDURE AND CHEMICAL METHODS

In the analysis of potatoes for their ascorbic acid content, the prob- m of representative sampling has often been overlooked or ignored. n the case of raw potatoes, grating or slicing and mixing, preliminary sampling increases the possibility of formation of dehydroascorbic cid and of irreversibly oxidized ascorbic acid. However, unless the istribution of ascorbic acid is uniform throughout the tuber, there an be no assurance that a small single sample from a tuber is repre- entative of the whole.

Preliminary experiments indicated that the distribution of ascorbic cid was not uniform throughout the tuber. All of the Maine-grown otatoes were sampled by the following method: Four wedge-shaped ections, weighing between 12 and 20 gm., were cut from the center the outer edge from each potato. One of these sections was cut om the stem end, another from the bud end, and one from each side. he small size of many of the Chippewa potatoes made it possible to nalyze entire tubers as one sample, and the larger potatoes of this roup and of the Green Mountain potatoes bought on the local market ere of such size that they could, in most cases, be represented accu- itely by two samples instead of four. When fewer than four samples ere cut from a potato, care was taken that the wedges selected for nalysis should represent both the stem and bud end of the tuber.

The ascorbic acid in raw potatoes was extracted by grinding each mple with 12 gm. of acid-washed sand under three successive por- ons (20 to 30 ml. each) of an aqueous solution containing 8 percent f acetic and 2 of metaphosphoric acid (*6, 11, 14, 15*). The three xtracts, separated from the pulp by centrifugation, were combined nd made up to 100 ml. Two 25-ml. aliquots of each were titrated ith 2-6-dichlorophenol-indophenol according to the method of Till- ians, Hirsch, and Hirsch (*24*) as modified by Bessey and King (*2*). ll reagents were made up with glass-distilled water and all glassware as rinsed with glass-distilled water before use.

The average of the ascorbic acid content of all the samples of a single otato was taken as its ascorbic acid value and from 5 to 10 potatoes ere used in each series of determinations. The analyses of raw po- itoes were made on pared tubers whenever paring was introduced reliminary to the cooking method; in all other cases analyses were on npared tubers.

Cooking causes a gelatinization of the starch in potatoes, and after ne cooked tissue is ground with acid and sand, centrifugation yields a ery cloudy suspension. It was found, however, that breaking up ne potato tissue with a stirring rod and mixing it thoroughly with the cid resulted in the removal of essentially the same percentage of the otal ascorbic acid with each extraction as when grinding with sand as employed (table 1). Also the resulting extracts were clearer and ne end points at titration easier to distinguish. Only a fraction of ne total ascorbic acid was removed by each extraction, and further xperiments showed that with the weight of sample and size of cen- rifuge tube (50 ml.) used, 12 washings were necessary to remove all f the ascorbic acid from cooked potato tissue. However, six extrac- ons constantly removed very close to 90 percent of the ascorbic cid present and at the same time the volume could be confined to 00 ml. As shown in table 1, this difficulty of removal of ascorbic cid was not encountered when raw potatoes were analyzed.

TABLE 1.—*Extraction of ascorbic acid from raw and cooked potato tissue*

Extractions (number)	Proportion of total ascorbic acid removed at end of each ex-traction					
	Cooked tubers,[1] samples—				Raw tubers, sa-ples—	
	A	B	C	D	E	F
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Perc*
1	24.2	24.2	27.8	26.9	82.3	
2	49.3	53.4	56.5	55.8	95.4	
3	65.6	70.9	69.7	69.4	99.2	
4	76.3	81.7	79.4	78.2	100.0	1
5	83.1	88.1	87.2	85.2		
6	90.5	93.5	93.7	92.4		
7	94.0	96.4	96.5	96.4		
8	97.2	98.4	98.4	98.4		
9	[2] 100.0	[2] 100.0	[2] 100.0	[2] 100.0		

[1] Samples A and B were ground with sand in the second, third, and fourth extractions. The remair extractions of these samples and all extractions of samples C and D were made without sand as describe the text.

[2] The titration value for the ninth extraction was so small that for practical purposes 100-percent extract was assumed.

The following procedure was employed in the analysis of cook potatoes: Centrifuge tubes containing about 20-ml. portions of t extracting medium were weighed and chilled in an ice bath. Fo wedge-shaped samples were taken as soon as possible after the potato were cooked and each sample was cut immediately into small piec and dropped into the cold acid. The tubes were then reweighed determine the weight of the sample. Extraction was carried out wi six successive portions of the mixture of acids, the potato tissue bei thoroughly broken up and mixed with each fresh portion of aci Centrifugation for 4 minutes at high speed gave a fairly clean separ tion of liquid and solid. The combined extracts were made up 100 ml. and two aliquots of each sample were titrated.

In consideration of the results presented in table 1, the values f ascorbic acid so obtained were taken to be 90 percent of that prese in the samples and the total amount was accordingly estimated c this basis. Finally, taking into account the loss or gain in weight c cooking, the values were readjusted to the basis of the raw weight the potato.

COMBINED ASCORBIC ACID

McHenry and Graham (*13*) and others have reported the presen of a protein ester or "bound" ascorbic acid in potatoes. This con pound is said to be soluble in water and insoluble in the ordina ascorbic acid extractants, but capable of hydrolysis on heating or c treatment with 1 percent hydrochloric acid to free the soluble ascorb acid.

Since both Green Mountain and Irish Cobbler potatoes are r ported (*19*) to contain large quantities of this ester, various attemp were made to determine its presence. The residues remaining aft the usual acid extraction of the raw potato tissue were treated with percent hydrochloric acid according to Reedman and McHenry directions (*19*). Several variations in time and temperature wer used. In no case was there any additional titration value as a resul of this treatment. The residue remaining after thorough extractio

of the potato tissue with trichloroacetic acid—a better protein precipitant than the acetic-metaphosphoric acid mixture—gave the same negative reaction to hydrolysis. This agrees with the results reported by Bessey (*1*) of similar experiments on Green Mountain and Irish Cobbler potatoes. Fujita and Ebihara (*5*) have reported recently that there are some grounds for believing the ascorbic acid in plant tissue to be partially bound to protein. They state, however, that this "bound" ascorbic acid is readily converted to the free form in the presence of metaphosphoric acid and is thus completely removed from the tissue by ordinary extraction procedure.

DEHYDROASCORBIC ACID

At various times during the course of the experiment, portions of the extract were treated with hydrogen sulfide for 20 to 30 minutes as a first step toward determining whether any dehydroascorbic acid was present. No increased reducing activity could be determined except in one case. Then it was not certain that all of the hydrogen sulfide had been removed. Bessey (*1*) found a small amount of dehydroascorbic acid in both Green Mountain and Irish Cobbler potatoes after storage. He recommends longer contact with hydrogen sulfide in a slightly less acid medium, which may account for the difference in his results.

EFFECT OF METHOD OF COOKING ON ASCORBIC ACID CONTENT OF POTATOES

In order that the potatoes cooked by different methods should attain the same stage of "doneness," cooking was discontinued when the internal temperature measured at the center of the tuber had reached 96° C. In each individual cooking, potatoes of approximately the same weight were used. A thermometer was inserted with the bulb at the center of one potato (two in baking) of each lot, with the exception of those prepared in the pressure saucepan and by "waterless" cooking. In the latter instances, the time required to bring a given weight of potato to the required temperature was determined by preliminary experiment, and this time was adhered to in the remainder of the tests. Potatoes without thermometers from each cooking lot were used for analysis. In the study of any given cooking method the tubers analyzed raw were of the same weight as those which were analyzed after cooking. The results of these analyses are given in table 2 in which the data showing the effect of each method of cooking upon the ascorbic acid content of potatoes are summarized.

BAKING

Three or four unpared Irish Cobbler potatoes weighing between 200 and 250 gm. were prepared in an oven maintained at a temperature of 215° C. The time required varied from 47 to 52 minutes. The potatoes lost 15 to 17 percent in weight and the average loss of ascorbic acid, calculated on the basis of raw weight, was 15 percent.

STEAMING

Three unpared Irish Cobbler potatoes weighing between 153 and 180 gm. were used for each cooking. They were cooked in a covered

steamer over 4 quarts of rapidly boiling water. This method require from 33 to 39 minutes except in one case when the time extended t almost 51 minutes. There was usually a gain in weight on the orde of 1 gm. per potato. No ascorbic acid was recovered in the water About 10 percent of the ascorbic acid originally present in the raw potatoes was destroyed.

TABLE 2.—*Ascorbic acid losses in cooking Irish Cobbler and Green Mountai potatoes*

Variety and cooking method	Raw potatoes		Cooked potatoes			Ascorbic acid recovery in—		Ascorbic acid destruction
	Tubers analyzed	Ascorbic acid per 100 gm. [1]	Tubers analyzed	Ascorbic acid per 100 gm. (raw basis) [1]		Cooked potato	Cooking liquor	
				In potato	In cooking liquor			
Irish Cobbler, stored:	*Number*	*Milligrams*	*Number*	*Milligrams*	*Milligrams*	*Percent*	*Percent*	*Percent*
Baked	5	10.6±0.21	5	9.0±0.30		85		15
Steamed, unpared	10	10.1±0.19	8	9.1±0.14		90		10
Boiled, unpared	10	10.1±0.19	8	9.1±0.21		90		10
Steamed in pressure cooker, pared	6	9.7±0.15	6	8.2±0.21	0.4±0.03	85	4	11
Boiled, pared	8	10.3±0.12	8	7.8±0.20	0.5±0.06	76	5	19
Green Mountain, "new":								
Boiled, pared	10	20.0±1.34	10	16.1±0.92	2.3±0.29	80	12	[2] 8
"Waterless" cooking, pared	10	17.3±0.73	11	15.8±0.47		91		[2] 9

[1] The figures following the symbol ± represent the standard errors of the mean.
[2] The extent of destruction of ascorbic acid is not statistically significant.

BOILING

The potatoes were cooked by immersion in boiling, distilled water. The volume of liquid required to keep the potatoes completely covered in a 3-quart saucepan (7 inches in diameter) throughout the cooking period was calculated from the formula:

$$\text{Total weight of potatoes} \times 2.5 - 200 = \text{milliliters of water required.}$$

Potatoes were boiled pared and unpared. Three potatoes weighing between 140 and 180 gm. were used in each case. The unpared potatoes (Irish Cobblers) required from 33 to 40 minutes cooking time and gained about 1 gm. apiece in weight. They lost about 10 percent of their original content of ascorbic acid, none of which was found in the cooking liquid.

The pared potatoes required from 31 to 37 minutes for cooking. The Irish Cobbler potatoes gained from 2 to 6 gm. in weight and there was very little sloughing. The potatoes contained 76 percent of their original ascorbic acid and an additional 5 percent was recovered in the cooking liquid so that the destruction of the vitamin was 19 percent.

The "new" Green Mountain potatoes were more mealy than the Irish Cobblers, which had been stored, and there was a great deal more sloughing during cooking. Nevertheless these potatoes retained 80 percent of their ascorbic acid value. The cooking liquid contained another 12 percent. The average destruction of ascorbic acid in these potatoes was 8 percent, but because of the magnitude of the

standard error, this difference cannot be regarded as significant. If the ascorbic acid present in the cooking liquid were disregarded the loss would be 24 percent in the Irish Cobbler and 20 percent in the Green Mountain potatoes.

PRESSURE COOKING

For the pressure-cooking experiment a 3-quart pressure cooker made of cast aluminum was used. The cover, which was automatically steamtight, contained a vent pipe to which a weight was fitted so that pressure could be maintained at 15 pounds. It was possible to cool the whole cooker very rapidly by placing it in a pan of cold water so that cooking was stopped almost instantly when the utensil was removed from the flame.

Two Irish Cobbler potatoes weighing between 100 and 114 gm. were cooked at one time. They were placed on a rack in the cooker and one-fourth cup of hot water was added. Two minutes were required for the pressure to reach 15 pounds and it was maintained at this point for 15 minutes, giving a total cooking time of 17 minutes. The pressure was released in 10 to 15 seconds.

The gain in weight for different samples of potatoes varied from zero to 4.5 gm. They retained on an average 85 percent of their ascorbic acid value and an additional 4 percent was recovered in the small amount of liquid remaining at the end of the cooking period, so that the destruction of vitamin C was 11 percent, with a loss of 15 percent if this liquid was discarded.

"WATERLESS" COOKING

Four "new" Green Mountain potatoes weighing between 95 and 135 gm. were prepared at one time in a 1½-quart, heavy aluminum, covered pan. They were washed after paring, placed in the pan, rinsed in cold water, and the water poured off so that the potatoes remained moist. During the first half of the cooking period the temperature inside the pan was maintained slightly below the boiling point so that the formation of vapor was slow and its escape from the container not apparent. Thereafter the temperature was lowered and for the remainder of the cooking period it was maintained at 84° to 86° C. An indicator in the cover of the pan made control possible.

The total cooking period was 64 minutes. At the end of cooking there was no remaining liquid, the potatoes were very lightly browned at the points of contact with the pan, and there was a slight skin formation. They lost from 3 to 5 gm. apiece in weight and an average of 9 percent of their ascorbic acid content. In view of the magnitude of the standard error, this loss of ascorbic acid cannot be regarded as significant.

RELATION OF DEGREE OF MATURITY OF POTATOES TO ASCORBIC ACID CONTENT

The Chippewa potatoes analyzed semiweekly for 6 weeks prior to maturity decreased in ascorbic acid content during that period from 25.5 to 20.1 mg. per 100 gm. (table 3). The ascorbic acid content of the potatoes did not change between the twelfth and fourteenth week after planting. At the end of the fourteenth week a decrease in

ascorbic acid occurred simultaneously with the appearance of a diseased condition in the tubers. For this reason, although only apparently healthy tubers were analyzed, it is uncertain whether or not the decrease in ascorbic acid value is attributable to the maturity of the vines. Lyons and Fellers (*12*) found no loss of ascorbic acid in maturing Green Mountain potatoes.

There was a wide variation in the size of the potatoes throughout the entire experimental period. Tubers ranging in weight between 12 and 193 gm. were analyzed. As far as possible, the entire size range was represented in each lot of potatoes analyzed and no relationship was found between the concentration of ascorbic acid present and the weight of the tuber.

TABLE 3.—*The ascorbic acid content of maturing Chippewa potatoes per 100 gm. of tuber and effect thereon of short periods of storage*

Period after planting (weeks)	Without storage [1]		Stored at 25.5° C. for [1]—					
			1 week		2 weeks		3 weeks	
	Tubers examined	Ascorbic acid content	Tubers examined	Ascorbic acid content	Tubers examined	Ascorbic acid content	Tubers examined	Ascorbic acid content
	Number	*Milligrams*	*Number*	*Milligrams*	*Number*	*Milligrams*	*Number*	*Milligrams*
12	25	25.5±0.74						
13	10	26.4±1.15	12	20.6±0.47	7	19.2±1.50	10	17.0±0.76
14	13	25.9±1.05			7	17.8±0.69	10	17.0±0.76
15 [2]	10	22.2±1.01					7	17.5±0.58
16	13	23.2±0.86			13	18.1±0.60		
17 [3]	14	20.1±0.69	7	18.7±1.05				

[1] Figures following the ± symbol show the standard error.
[2] Fusarium wilt was well developed.
[3] The vines were dead at this time.

EFFECT OF STORAGE ON ASCORBIC ACID CONTENT OF POTATOES

Olliver (*16*) reported a loss of 50 percent of the ascorbic acid from new potatoes after 15 days of storage and Pett (*18*) a loss of over 50 percent after 20 to 30 days. In the present study there was also a marked decrease in the ascorbic acid content of stored potatoes, very rapid during the first few weeks after harvesting, and more gradual thereafter. The immature Chippewa potatoes lost 22 percent of their ascorbic acid after 1 week's storage at 25.5° C. and 36 percent in 3 weeks (table 3).

"New" Green Mountain potatoes bought on the market decreased in ascorbic acid content from 20.0 to 17.3 mg. per 100 gm., a loss of 14 percent in about 10 days at 25.5° C. These potatoes had probably been harvested for at least a week before the first analysis for ascorbic acid was made.

The Maine-grown Green Mountain potatoes lost about 30 percent of their ascorbic acid in the first month of storage at 15.5° C. (fig. 1).

Unfortunately no figures were obtained for the Irish Cobbler potatoes during the first period of storage, but the close agreement of the curve with that of the Green Mountain potatoes for the remainder of the 15.5° storage period suggests the probability of similar behavior at the beginning. Green Mountain potatoes stored at 15.5° for 5

nonths had an ascorbic acid content of 10.1 mg. per 100 gm., a loss of nearly 50 percent of their original value. Irish Cobblers stored for the same period and at the same temperature contained 9.9 mg. per 100 gm.

It is interesting to note that in these potatoes the rate of loss of ascorbic acid at 15.5° and 25.5° C. was apparently independent of the variety of the potato, but dependent, to some extent at least,

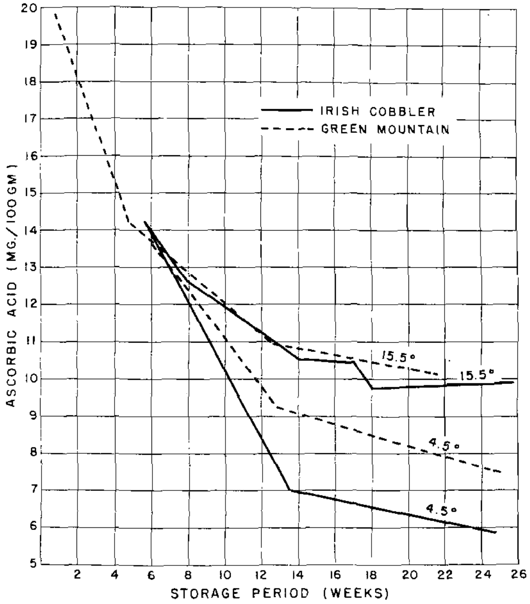

FIGURE 1.—Ascorbic acid content of Irish Cobbler and Green Mountain potatoes during storage at 15.5° and 4.5° C.

upon the ascorbic acid content of the tuber. The variation in ascorbic acid content from tuber to tuber was much smaller in potatoes that had been stored for a short period than in immature and new potatoes. This approach to uniformity during storage could only have been brought about if the loss of the vitamin were greater during the same period from tubers of high ascorbic acid content than from those of lower ascorbic acid content.

When Irish Cobbler and Green Mountain potatoes were placed in storage at 4.5° C. after a preliminary period of 6 weeks at 15.5° the loss of ascorbic acid was more rapid in both varieties as compared with the loss at 15.5°. At the end of the storage period (25 weeks) the ascorbic acid content of the Green Mountain potatoes had dropped to 7.5 mg. per 100 gm. and that of the Irish Cobblers to 5.9 mg. per 100 gm. Apparently the interference with the physiological processes caused by lowering the temperature has a marked effect upon ascorbic acid.

From these results it would seem that the storage of potatoes in cellars where the temperature approaches the freezing point during the winter would cause a greater loss of ascorbic acid than storage in warmer cellars. However, Kröner and Steinhoff (*8*), who kept potatoes in such a storehouse, found that the ascorbic acid content reached a low value and then increased steadily during the spring, following the rise in the temperature of their storehouse.

In view of the increase noted by these authors and of the data presented here, it seems possible that the detrimental effects of storage at a low temperature upon the ascorbic acid content of potatoes might be offset to some extent by later storage at a higher temperature.

DISTRIBUTION OF ASCORBIC ACID IN THE POTATO TUBER

It has been repeatedly stated (*7, 12, 17*) and apparently is usually assumed that the distribution of ascorbic acid in the raw potato is uniform or nearly so, although Rudra (*21*) found a higher concentration in the skin than in the flesh of new potatoes. Some of the potatoes used in the present study were examined after a period of storage, and it was found that, although there was a tendency for the concentration to be higher in the cortical layer, the difference between it and the medullary layer was slight.

When wedge-shaped samples were taken from various parts of the potato it was noticed that the concentration of ascorbic acid was always higher in the bud end of the tnber than in the stem end. This variation led to the method of sampling previously described, that of taking four wedge-shaped samples from each potato analyzed: One each from the stem and the bud end, and one from each side.

Figure 2 shows the relationship between the ascorbic acid content of these various wedge-shaped sections of Green Mountain and Irish Cobbler potatoes during storage at 15.5° and 4.5° C. Each point on the curves represents the average of the values of from 5 to 10 tubers.

A similar distribution was present in the cooked potatoes both pared and unpared. This shows that there was little diffusion of the vitamin during cooking. The sharp rise shown in the curve for the bud end of Irish Cobblers stored at 15.5° C. occurred just before the removal of all of the sprouts and the abrupt decline immediately after this treatment. No analyses were made of the Green Mountain potatoes at this time.

With regard to the potatoes studied later, the immature Chippewas showed practically no difference in the distribution of ascorbic acid in the various sections, although there was a slight tendency toward a higher concentration in the bud end as they approached maturity. The new Green Mountain potatoes bought on the market showed only a slight tendency to have a higher concentration of ascorbic acid in the

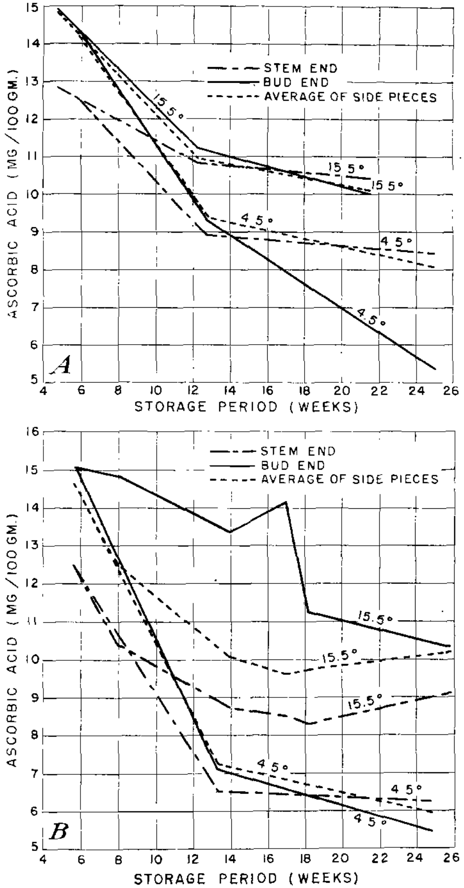

FIGURE 2.—Distribution of ascorbic acid in potatoes stored at 15.5° and 4.5° C.: *A*, Green Mountain; *B*, Irish Cobbler.

bud end than in the stem end when purchased. This difference became very apparent by the end of the first week and increasingly more pronounced thereafter.

DISCUSSION OF RESULTS

The results of the present study indicate that the vitamin C value of potatoes is more dependent upon the time that elapses after harvesting before the potatoes are consumed and the temperature at which they are stored during that time than upon the method of preparation for consumption. The method of cooking most commonly employed, the boiling of pared potatoes, causes the greatest loss in ascorbic acid content, particularly since the cooking liquor is usually discarded. Losses due to other methods of cooking are somewhat smaller and fairly comparable. However, the decrease in the ascorbic acid content of potatoes during the first few weeks after harvesting exceeds even that which would be produced by the most destructive of the cooking methods.

Data presented here in regard to the effect of cooking upon the ascorbic acid content of potatoes is in general agreement with that of Scheunert, Reschke, and Kohlemann (*22*) and Wachholder, Heidinger, Grieben, and Köhler (*26*). The former reported the effect on the ascorbic acid content of steaming unpared potatoes and the latter that of steaming unpared potatoes and of boiling both unpared and pared potatoes. Much greater losses in ascorbic acid than were found here have also been reported, while some investigators have noted similar losses with some of the methods of cooking and gains with others. No such gains were found in the present investigation and no evidence was obtained in support of any of the theories advanced to explain these gains.

The apparent inconsistencies of the gains reported for ascorbic acid in cooked potatoes suggest the operation of an accidental factor. If, for example, boiling pared potatoes causes an an apparent increase in ascorbic acid content, boiling unpared potatoes should have the same effect. However, if the type of distribution of ascorbic acid shown by Irish Cobbler potatoes at 15.5° C. were present but unrecognized, it is easy to see that the accidental selection of a disproportionately large number of samples from the bud end of the tubers would result in a relatively high ascorbic acid content as estimated from this group of samples. If, in addition, these samples represented cooked potatoes, the high value obtained would be thought to be a result of cooking rather than of sampling.

Another essential factor in the determination of the actual effect of a cooking method upon the ascorbic acid content of potatoes is the elimination of the effect of storage. This is particularly important when the potatoes are studied soon after being harvested since the rate of decrease in their ascorbic acid content is extremely rapid at this time. Unless determinations of the ascorbic acid content of both raw and cooked potatoes parallel each other in point of time, the result obtained for the effect of a cooking method may be very erroneous. In addition, the variation in ascorbic acid content from tuber to tuber is much greater soon after harvesting than it is after a period of storage. Consequently the sampling of an adequate number of tubers is imperative.

SUMMARY

In sampling potatoes for the determination of their ascorbic acid content, all sections of the tuber were represented in proportion to their presence in the potato. The vitamin was completely extracted from raw potato tissue more easily than from the cooked tissue. Six successive extractions removed almost a constant percentage of the total ascorbic acid from the latter and a factor was employed to calculate the total.

There was no evidence of the presence in potatoes of a "bound" ascorbic acid insoluble in the extracting medium employed. No dehydroascorbic acid was found in the potatoes analyzed.

New Green Mountain potatoes were used in a comparison of the effects of boiling and "waterless" cooking upon the ascorbic acid content of potatoes. The variation in the ascorbic acid content from tuber to tuber in the new potatoes was so great that the losses obtained as a result of cooking these potatoes could not be regarded as significant.

Irish Cobbler potatoes stored at 15.5° C. were used to study the effect of pressure cooking, baking, boiling, and steaming upon the ascorbic acid content. Steaming and boiling unpared potatoes were the most conserving of vitamin C. Baking and pressure cooking caused slightly larger losses of ascorbic acid, while boiling pared potatoes was least conserving of the vitamin. However, the maximum loss of ascorbic acid due to a cooking method never exceeded 25 percent.

Chippewa potatoes decreased in ascorbic acid content during maturation. Because of the development of fusarium wilt during the growing period it was uncertain whether the effect shown was caused by maturity or disease.

All three varieties of potatoes lost ascorbic acid during storage. At 15.5° C. the losses were most rapid during the first few weeks of storage and became more gradual thereafter until, at the end of 26 weeks of storage, the ascorbic acid content had nearly reached a plateau value. Storage of a lot of Green Mountain and Irish Cobbler potatoes at 4.5° caused their ascorbic acid content to drop below that of a similar lot kept at 15.5°.

Ascorbic acid was not distributed uniformly throughout the tuber. Moreover the relative distribution of ascorbic acid differed in Green Mountain and Irish Cobbler potatoes. It was affected by the temperature at which the tubers were stored and was subject to continuous change during the period of storage.

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STIMULATION OF GROWTH IN JUVENILE MANGOSTEEN PLANTS [1]

By Claud L. Horn

Associate horticulturist, Puerto Rico Experiment Station of the United States Department of Agriculture

INTRODUCTION

Much of the literature pertaining to the mangosteen, *Garcinia mangostana* L., laments the fact that this much-desired tropical fruit tree is so difficult to grow. The main difficulty lies in the often-mentioned poor vigor of the young plants. In the nursery of the Puerto Rico Experiment Station of the United States Department of Agriculture at Mayaguez, there are some plants as much as 5 years of age which are no more than 6 inches high. Most plants fail to survive the juvenile stage, but the few that do survive develop to maturity in a manner much the same as seedlings of other tree species. The root system of a juvenile plant consists of a slender taproot having corky bark and little, often none, of the more active lateral system of smaller roots. In general, the poor development of mangosteen plants has been attributed to this poor root system.

Oliver,[2] working on the problem of mangosteen grafting under greenhouse conditions in Washington, D. C., about 1910, found that poor mangosteen plants could be approach-grafted on *Garcinia tinctoria*, a close relative. After such grafting, the mangosteen became vigorous, and new roots developed from the base of the mangosteen stem where the root system had been severed after union with the *G. tinctoria* stock had been accomplished. The new mangosteen root system continued to develop and ultimately the mangosteen reestablished itself upon its own roots and the *G. tinctoria* root system perished

Working with plants of *Aleurites fordii* whose growth bad entirely ceased, Bonner and Greene [3] obtained almost twice as much total shoot elongation and a more luxuriant root system in plants supplied with vitamin B_1 than in similarly treated plants without vitamin B_1. According to Bonner,[4] vitamin B_1 is essential for root growth and in the presence of light it is normally formed in the leaves. The root of a normal seedling may receive its supply of this vitamin either from the green leaves or from the seed where it is normally stored in sufficient quantity for the needs of the young plant. The favorable behavior of the grafted mangosteen plants of Oliver might be attrib-

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Key No. P. R.-8

uted to a vitamin B_1 deficiency in the mangosteen seed being supp mented from a well-supplied root system or from the active leaves the *Garcinia tinctoria* stock plant.

With knowledge of this and of the results of Bonner and Gre with *Aleurites fordii* as a background, the writer conducted an exp ment at the Puerto Rico Experiment Station to determine whet supplementary growth substances may be obtained by mangost seedlings from a water extract of brewer's yeast to give the stimulat needed for satisfactory growth. Brewer's yeast is known to be a go source of vitamin B_1; however, this vitamin is not the only grov substance which roots can obtain from such yeast.

EXPERIMENTAL METHODS

Seeds were planted individually in cylinders 4 inches in diam by 1 foot deep filled with dead sphagnum moss; the seeds were fir embedded in the moss. Moisture was added only in nutrient so tion. Fresh mangosteen seeds germinate slowly but without difficul

The experiment consisted of two treatments each containing plants. All plants were irrigated once weekly with an excess nutrient solution so that a flushing action was thereby accomplish The nutrient solution used consisted of White's [5] solution with o tenth of 1 percent of raw cane sugar to which were added the tr elements through a modification of Hoagland's mixture.[6] One of two lots of plants received a water extract of brewer's yeast in addit to the combination of White's solution and Hoagland's A to Z mixt and raw cane sugar. The nutrient solutions were prepared in 18-li quantities. Stock water extracts of yeast were prepared by heat for 96 hours, at a temperature of 100° to 103° C., crown-capped bot each containing 7.2 gm. of Anheuser-Busch strain K yeast suspen in 200 ml. of distilled water. After standing for 3½ months, t preparation had a pH value of 5.17. Fifty milliliters of the coo supernatant fluid thus obtained was used in the preparation of liters of the nutrient solution for the yeast-fed plants.

The plants were grown for 7 months under uniform moisturepr protective sashes in the open nursery yard where uniform shade provided by light cheesecloth. During the last 8 months of the periment the plants were grown in a greenhouse where shade was p vided by light cheesecloth. Temperatures were higher in greenhouse than in the nursery yard.

EXPERIMENTAL RESULTS

The advance in development of the plants that received ye extract, as illustrated by figure 1, *A*, over the plants that did receive yeast, as illustrated by figure 1, *B*, distinctly shows the be ficial effect of yeast upon the development of the mangosteen pla during their first 10-month growth period. An increase in grov during this most critical period was obtained when the plants

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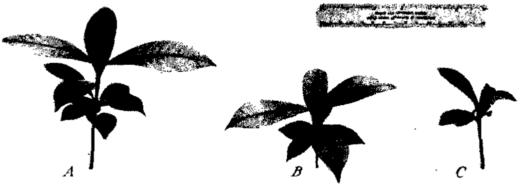
[6] ROBBINS, WILLIAM J., and SCHMIDT, MARY BARTELY. GROWTH OF EXCISED ROOTS OF THE TOM Bot. Gaz. 99: 671–728. 1938.

hagnum moss irrigated with nutrient solution were given water tract of brewer's yeast. As leaf area increased, differences due to ast-extract stimulation diminished. At the seedling age of 10 onths the increase due to yeast extract was 75.8 percent, while at months it was 46.8 percent. If the effective principle in the yeast tract was vitamin B_1, this decrease in growth rate was as should be



URE 1.—Typical plants of *Garcinia mangostana* grown under different treatments. *A* and *B* were grown in dead sphagnum moss irrigated with nutrient solution. *A* received in addition a water extract of brewer's yeast. *C* is a plant of comparable age grown in garden soil only.

pected, for this vitamin is developed in the leaves and translocated the roots. Table 1 presents the average leaf areas and the rcentage increase for the two treatments together with the standard viations and coefficients of variability for the measurements.

BLE 1.—*Average leaf area and other data pertinent to the evaluation of differences between mangosteen plants grown with and without yeast extract*

Treatment	Age of plants	Average leaf area	Increase due to yeast	Standard deviation	Coefficient of variability
	Months	*Cm.²*	*Percent*		*Percent*
rient solution	10	97.94		8.95	9.1
	15	252.18		109.34	43.4
rient solution with yeast	10	172.20	75.8	12.23	7.1
	15	370.12	46.8	164.71	44.5

That the average leaf-area difference between the two treatments both age levels was due directly to the effect of the addition of ast is shown by the fact that this difference exceeded its standard or by an amount sufficient to assure an accuracy well beyond the percent point where the odds are 99 to 1.

All the plants in this experiment, whether they received yeast tract or not, grew much more rapidly than those planted in good rden soil. The typically poor development of mangosteen plants own in good garden soil is illustrated by figure 1, *C*, a plant from a

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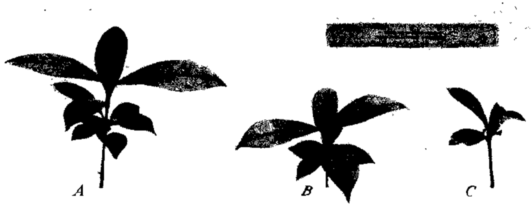

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group not within this controlled experiment and a few weeks old than *A* and *B*. Whether the increase in growth of the plants grow in sphagnum moss and receiving only nutrient solution over tho grown in soil was due to the nutrient solution or to the sphagnum mo or both was not brought out in this experiment. The profoun increase in growth is, however, highly insignificant

SUMMARY

Normal growth in young mangosteen plants is so poor that fe develop beyond the juvenile stage. This poor growth is the greate limiting factor in the development of this tropical fruit crop. Th few mangosteen plants that do survive the weak juvenile period co tinue growth to maturity much as do plants of any other vigorousl growing tree species. Plants grown in dead sphagnum moss an irrigated with nutrient solution having yeast extract develop 75.8 percent more leaf area within 10 months than similarly treate plants receiving no yeast extract. Growth stimulation by yea extract was greater in the first 10 months than in the succeedin 5 months. Such behavior should be expected if the effective princip in the yeast extract is vitamin B_1, for, normally, a plant provided wit leaf area equal to that of these 10-month-old plants is capable o producing a sufficient quantity of vitamin B_1 for its current growt needs. Correlation is seen between this early stimulation by yeas extract and the growth-stimulating effect Oliver obtained when man gosteen was grafted on the more vigorous *Garcinia tinctoria*. Growt in all plants within this experiment whether or not they received yeas extract was profoundly better than that of plants grown in goo garden soil only.

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EFFECT OF EXCHANGE SODIUM ON THE MOISTURE EQUIVALENT AND THE WILTING COEFFICIENT OF SOILS [1]

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INTRODUCTION

Laboratory research by numerous investigators has shown that sodium adsorbed by the clay complex of soils increases dispersion, pH values, swelling, osmotic imbibition, migration velocity, apparent density of puddled soils, and the hardness of dry aggregates; and lowers heat of wetting, sticky point, and permeability.

The moisture equivalent (*3*, *4*)[5] and wilting coefficient (*5*), more than any other soil constants, have come into extensive use as reference points for the water relations of soils and plants. Sharp and Waynick (*17*), Joseph (*10*), Anderson (*1*), and others (*20*) have been in agreement in finding that adsorbed sodium markedly increases the moisture equivalent. Veihmeyer and Hendrickson (*21*) have concluded, however, that the change, when any, can be attributed to soil puddling. In connection with all results it can be appropriately mentioned that the magnitude of the effect that adsorbed sodium has on soil characteristics is related to the amount and character of the clay of the soil, to the extent to which other ions are replaced by sodium, and to the extent to which flocculating electrolytes are removed.

No one has reported on the effects of adsorbed sodium on the availability of moisture to plants in the wilting range, and, so far as the writers have been able to learn, no investigations have been conducted.

In agreement with laboratory findings, it has been extensively observed that when lands are irrigated with water containing a high proportion of sodium relative to the concentrations of other bases the permeability tends to be reduced. Accompanying the effects of adsorbed sodium on permeability are other adverse consequences, such as the accumulation in the root zone of the salt constituents of irrigation waters, increased erodibility, the loss of good tilth, and consequent unsatisfactory seedbeds. It is a common observation that

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[5] Italic numbers in parentheses refer to Literature Cited, p. 424.

soils with much adsorbed sodium are less permeable to rain water than they are to saline irrigation waters.

The expression "percent sodium," as used by Eaton (7) to designate the relation

$$\text{Sodium-total base ratio} = \frac{\text{milliequivalents Na} \times 100}{\text{milliequivalents of total bases}},$$

has come to be regarded as an important water-quality characterization. Because of the diversity of factors that bear on permeability and exchange reactions and the quantitative relationships involved, it has not been possible to closely delimit the concentrations and proportions of sodium in natural waters that should be avoided or designated as undesirable.

The relationships between adsorbed sodium and moisture equivalent that are reported on in this paper were investigated for the purpose of exploring the possibility of utilizing the moisture equivalent as an index to sodium-induced dispersion. As a part of the inquiry, comparisons were made between calcium-treated and sodium-treated soils with respect to (1) the effect of the acceleration rate of the centrifuge on the moisture equivalent; (2) the migration and segregation of sand, silt, and clay in dispersed soils during centrifuging; (3) the distribution of moisture in Ca soils and Na soils after centrifuging; (4) the effect of flocculation by electrolytes on the moisture equivalent of Na soils; and (5) the quantity of sodium that significantly affected moisture equivalent values when Ca and Na soils were mixed in different proportions. Finally, moisture equivalent and hydroscopicity comparisons were made between soils saturated with calcium, magnesium, sodium, and potassium. This was done for the purpose of determining whether the effects of potassium most closely resembled those of calcium and magnesium or those of sodium.

The relationships between the adsorbed sodium and moisture availability were investigated with 12 soils, both at the wilting coefficient and at the ultimate wilting point. The resulting data, together with the moisture equivalent values, are graphically presented with the use of a pF scale.[6] (See fig. 3.)

DESCRIPTION OF SOILS, DEFINITIONS, AND METHODS

The source, classification, and certain physical constants of the 12 soils used for these investigations are reported in table 1.

MOISTURE EQUIVALENT

The moisture-equivalent values represent the percentage of moisture remaining in 30-gm. samples of soil that had been saturated for 24 hours, drained for 30 minutes, and then centrifuged for 30 minutes in standard cups in a standard moisture equivalent centrifuge drum operated at a rotational speed of 2,440 r. p. m.[7] The centrifuge came to full speed in 15 seconds. Any free water remaining on the surface of the soil after centrifuging was removed by inverting the cups for several minutes and then wiping their inside walls with blotting paper.

[6] The pF value of soils is the logarithm of the equivalent capillary tension expressed in centimeters of water column.

[7] The centrifuge drum was belt-driven from a 1-hp. polyphase induction motor following a principle of operation worked out by E. S. Babcock, of Riverside, Calif. By the use of a large motor with a low connected load, the speed of the drum is determined alone by the cycles of the alternating current and the ratio of the two pulleys. The centrifuge drum was mounted on the vertical pulley, within which were two roller bearings set on a 1¼-inch shaft anchored in a concrete pedestal. With this machine the acceleration rate is rapid, though it is possible to reduce it by installing an autotransformer (choke coil) in the line.

Soil No.	Soil type	Location	Moisture equivalent[1]	Xylene equivalent[2]	Wilting coefficient[3]	Apparent density[1]	Exchange capacity per 100 gm.	Mechanical analysis of composite[1,4] Sand, 2–0.05 mm.	Silt, 0.05–.002 mm.	Clay, 0.002 mm.
						Gm./Cc.	*Milli-equivalents*	*Percent*	*Percent*	*Percent*
279	Gold Ridge fine sandy loam	Sonoma County, sec. 35, T. 6 N., R. 9 W	10.6	4.4	3.6	1.31	3.34	71.3	22.2	6.5
1	Sierra loam	Riverside County, sec. 22, T. 2 S., R. 5 W	8.8	4.3	3.7	1.44	4.82	60.7	33.4	5.9
4	Yolo loam	Ventura County, sec. 26, T. 4 N., R. 20 W	11.5	4.3	4.7	1.43	7.13	64.6	26.2	9.2
2	Hanford sandy loam	Kern County, sec. 8, T. 31 S., R. 30 E	11.7	4.2	5.5	1.36	8.54	72.6	17.8	9.6
6	Yolo loam	Ventura County, sec. 28, T. 3 N., R. 22 W	12.4	5.3	5.7	1.46	8.02	62.2	28.7	9.1
3	do	Ventura County, sec. 2, T. 3 N., R. 21 W	14.1	4.0	5.8	1.41	9.32	50.7	37.3	12.0
9	Antioch clay loam	Stanislaus County, sec. 31, T. 3 S., R. 7 E	18.0	7.9	10.2	1.37	11.05	38.0	36.3	25.7
5	Yolo silt loam	Ventura County, sec. 19, T. 3 N., R. 21 W	20.6	7.5	9.8	1.27	16.46	40.5	41.1	18.4
10	Antioch clay	Stanislaus County, sec. 31, T. 3 S., R. 7 E	21.0	11.1	12.0	1.40	15.83	19.4	50.5	30.1
8	Yolo fine sandy loam	Ventura County, sec. 4, T. 2 N., R. 22 W	22.4	11.6	11.7	1.22	14.82	27.5	51.5	21.0
7	Rincon loam, silty phase	Ventura County, sec. 23, T. 3 N., R. 22 W	22.9	6.0	11.4	1.38	20.00	37.1	35.6	27.3
278	Aiken clay loam	Butte County, sec. 14, T. 22 N., R. 3 E	36.2	24.7	22.0	1.09	14.77	37.6	35.8	26.6

[1] Untreated soil.
[2] Means of determinations on washed, calcium-treated, and sodium-treated soils (percentage by weight).
[3] Ca soil.
[4] The mechanical analyses were made in accordance with the pipette method of the former Bureau of Chemistry and Soils, U. S. Department of Agriculture.

XYLENE EQUIVALENT

The xylene technique was the same as that used for the moisture equivalent, except that the samples were oven-dried just before they were wet with xylene. Xylene-equivalent measurements were made on washed, calcium-treated, and sodium-treated soils. The character of the adsorbed cation, in accordance with Joseph's (*10*) findings for soil clays, was without effect on the percentage of xylene held against a force of 1,000 times gravity. The greatest single departure between the xylene equivalents of any soil as treated with calcium and sodium was 2.6. The average of the values of the xylene equivalents was 12.9 for the 12 Ca soils and also for the 12 Na soils.

WILTING COEFFICIENT AND ULTIMATE WILTING POINT

The exhaustion of soil water that accompanies the wilting of plants, as recognized by Briggs and Shantz (*5*) and subsequently by others, is progressive, and extends through what is now termed the "wilting range." The fact that wilting does occur over a range of moisture percentages for a given soil adds to the difficulties associated with the selection of a uniform end point. The wilting coefficient, as here used, represents a degree of wilting from which only about one-third of the leaves of sunflowers recovered when the plants were placed in a dark, humid chamber overnight. Except as noted in table 10, each wilting coefficient and ultimate wilting point value is the average of six determinations.

Water and soil were alternately added (washed, Ca, and Na soils alike) as the cans were filled for the wilting coefficient measurements. After germination of the sunflower seeds additional water was added to the soil surface until a plant of the desired size was developed, after which the openings in the covers of the cans were closed with cotton. After the plants had been wilted and cropped, the soils were dried at 105° to 110° C. for 4 days. The soil of each can was then broken up and passed through a 2-mm. screen and rolled in a paper to remix. One sample was withdrawn from each wilting coefficient can for moisture equivalent measurement. Any moisture equivalent determination that appeared out of line was repeated with a new sample. The soil of the six cans of each treatment was then composited as stock samples for use in other parts of the investigation.

The "ultimate wilting point," a term suggested by Taylor, Blaney, and McLaughlin (*18*), is used in the present work to designate the moistnre content at which the rate of movement of water into a 15- to 30-cm. sunflower plant, after wilting, from a 600-gm. mass of soil was only sufficient to bring about the recovery of the terminal pair of leaves (1 to 2 cm. long) when the plant was placed overnight in the moist chamber. As this end point was approached the loss of water was a fraction of a gram per day, or in the order of 0.1 percent of the weight of the soil mass. The atmospheric conditions in the Riverside, Calif., greenhouse where the plants were grown were conducive to high transpiration rates. After recording the pot weights at the wilting coefficient, the same plants were reexposed in the greenhouse until the ultimate wilting point was reached.

The old leaves of plants on the majority of the Na soils deteriorated somewhat more rapidly than those on the Ca soils, and the death of leaves on the former plants was sometimes accompanied by marginal

burning. Equally good distributions of roots were observed in the Ca and Na soils. A further reference is made to the wilting measurements under soil treatments.

APPARENT DENSITY

Apparent density of untreated soils, as reported in table 1, represents the weight per unit volume of air-dry soil that had been passed through a 2-mm. sieve. The volume was measured in a 5 by 5 by 2.5 cm. box. The box was filled and lightly tapped on the desk top and leveled off before weighing; the method of filling the boxes was as nearly alike as possible for all soils.

EXCHANGE CAPACITY

For the measurements of the exchange capacity, a 20-gm. portion of soil was digested with 250 cc. of neutral normal ammonium acetate and leached free of calcium with 500 cc. of neutral normal ammonium acetate. The excess ammonium acetate was then removed by leaching with neutral normal ammonium chloride followed by leaching with neutral methyl alcohol until the leachate was free from chloride ion. The adsorbed ammonia (NH_3) was distilled, in the presence of magnesia (MgO), into a 2-percent solution of boric acid and titrated with standard sulfuric acid. The values reported in table 1 are the means of washed, sodium-treated, and calcium-treated soils; the values were in close agreement.

PREPARATION OF SOILS

Two sets of soils were prepared for these investigations. The first set—designated washed, Ca, and Na soils—provided material for the comparisons of the effects of calcium and sodium on the moistnre equivalent and on the wilting coefficient and ultimate wilting point. The second set—designated Ca, Mg, K, and Na soils—was used only for the supplementary hydroscopicity and moisture equivalent measurements where the effect of potassium was of principal interest. Descriptions of the methods employed in preparation of the two sets of soils follow.

Washed, Calcium-Treated, and Sodium-Treated Soils, and Measurement of Exchange Sodium

Three 4-kg. portions of each of the 12 soils (table 1) were weighed into porcelain dishpans. The washed soils were successively treated with distilled water in parallel with the soils treated with calcium and sodium. The Ca and Na soils were treated three successive times with 3-liter portions of normal calcium chloride and sodium chloride solutions, respectively. Each suspension was stirred several times during the day and allowed to settle overnight; the supernatant solution was then decanted off and additional solution was removed with filter candles. The soils were then successively washed with 3-liter portions of distilled water until, in a final washing, the solutions contained less than 10 milliequivalents per liter of chloride ion. This washing required from 15 to 18 liters of water. To each of the 4-kg. portions of soil, from which all possible solution had been removed by the filter candles, 1 liter of Hoagland's nutrient solution was added. This solution contained 5, 5, 2, and 1 millimoles, respec-

tively, of calcium nitrate ($Ca(NO_3)_2$), potassium nitrate (KNO_3), magnesium sulfate ($MgSO_4$), and monopotassium phosphate (KH_2PO_4). The soils, still in the original pans, were then set in the sun until they had dried. After they had been passed through a 2 mm screen, they were wet to approximately the moisture equivalent with distilled water and frozen at −10° C. for 3 days and again air-dried and screened. Freezing left the soils reasonably friable. The dried aggregates of the Na soil, though easily broken, were harder than those of the washed or Ca soils. The soils as thus treated were used for the wilting coefficient and ultimate wilting point measurements.

Replaceable sodium concentrations in the Na soils were measured after the soils had been removed from the wilting coefficient cans. Total sodium was determined by the uranyl zinc acetate method in an aliquot of the ammonium acetate extract (see section headed Exchange Capacity, p. 405) from which organic matter and silica had been removed. From the total sodium so determined there was subtracted the sodium in solution in the soils as wet with water to three times the moisture equivalents found for the Ca soils. Aliquots of solution for these latter determinations were obtained by centrifuging four 50-gm. portions of each soil in glass tubes. It has been shown by Eaton and Sokoloff (*8*) that the apparent adsorbed sodium, as calculated by subtracting the sodium of aqueous extracts from the total obtained by ammonium acetate extraction, decreases as the soil-water ratio used for extraction is increased.[8] A number of the Na soils of this series as loosely placed in centrifuge cups and wet from below (the standard procedure) absorbed approximately three times the moisture equivalent of water of the Ca soils.

Electrical-conductivity measurements made on the foregoing centrifuge extracts of five of these soils gave values between 63×10^{-5} and 154×10^{-5} reciprocal ohms at 25° C., indicating the presence of between 6 and 15 milliequivalents of salt per liter of solution at this moisture content. This salt included the residual soluble material remaining after the sodium treatments, that of the nutrient solution which was added, and any salt brought into solution during the growth of the sunflowers, minus the salts taken up by the plants.

TABLE 2.—*Exchange capacity and exchange sodium in Na soils*

Soil No.	Exchange capacity per 100 gm.	Sodium in NH_4Ac extract per 100 gm.	Sodium in 3× moisture equivalent extract per 100 gm.	Exchange sodium	
				In soil per 100 gm.	Proportion of exchange capacity
	Milliequivalents	*Milliequivalents*	*Milliequivalents*	*Milliequivalents*	*Percent*
279	3.34	3.03	0.59	2.44	73.1
1	4.82	3.71	.69	3.02	62.7
4	7.13	5.33	1.37	3.96	55.5
2	8.54	5.54	1.10	4.44	52.0
6	8.02	6.07	1.19	4.88	60.8
3	9.32	7.12	.96	6.16	66.1
9	14.05	10.25	1.38	8.87	63.1
5	16.46	10.42	2.25	8.17	49.6
10	15.83	13.85	1.59	12.26	77.4
8	14.82	9.95	2.16	7.79	52.6
7	20.00	14.47	1.72	12.75	63.8
278	14.77	3.52	1.21	2.31	15.6

[8] Since this paper was written, Kelley (*11*) has published data confirming these findings of Eaton and Sokoloff. His data show for an Imperial Valley soil 5.1 m. e. of absorbed sodium per 100 gm. as determined on the basis of displaced solution, but only 0.6 m. e. on the basis of water extracts. The corresponding values for his Fresno soil 887 were 3.3 and 2.6 m. e., respectively.

As shown by table 2, the sodium chloride treatments did not saturate the soils with sodium and they were better suited, for this reason, to the purposes of the experiment. Neither Gedroiz (*9*) nor Ratner (*13*) was successful in growing plants in soils containing somewhat higher percentages of sodium. In all probability calcium was the principal base in the washed soils, since replacement proceeds in the calcium direction when soils with calcium carbonate are repeatedly treated with distilled water. The sequence of changes accompanying progressive leaching with distilled water of calcareous soils containing sodium (*8*) are (1) dilution of the aqueous phase and (2) a resultant replacement of some of the adsorbed sodium by calcium. As the process is continued, sodium of the aqueous phase is removed by leaching and new calcium comes into solution from calcium compounds, followed by further exchange of calcium for adsorbed sodium.

Soils Treated With Calcium, Magnesium, Potassium, and Sodium

The set of soils treated with calcium, magnesium, potassium, and sodium was given a uniform pretreatment with ammonium acetate and ammonium hydroxide to remove or reduce calcium carbonate and organic matter and thereby make possible a higher percentage saturation with the introduced bases. One-kilogram aliquots of each of the soils were suspended twiee in 4 liters of normal ammonium acetate, then in 4 liters of N/10 ammonium hydroxide, and again in 4 liters of normal ammonium acetate. Durmg each suspension the soils were recurrently stirred during the day (in the final ammonium acetate for 4 days), allowed to settle overnight, and then the supernatant solution was decanted and additional solution removed with filter candles. The foregoing treatments did not remove all calcium but none of the final solutions contained more than 10 milliequivalents per liter. The soils were dried, ground to pass a 2-mm. sieve, and divided into five 200-gm. aliquots, only four of which were used.

Each 200-gm. aliquot of each soil was treated three successive times with 800 cc. of normal salt solution ($CaCl_2$, $MgCl_2$, KCl, or NaCl, respectively), and then washed with distilled water, by suspension and stirring and the removal of solution with filter candles, until the chloride content of the suspending solution was less than 5 milliequivalents per liter. The potassium-treated and sodium-treated soils became highly dispersed during the washing with water and certain of them liberated some additional organic matter, but by the end of the washing little came into solution. The soils were finally air-dried, ground, and thoroughly mixed by rolling.

EXPERIMENTAL RESULTS

EFFECT OF STARTING ACCELERATION ON THE MOISTURE EQUIVALENT OF CALCIUM-TREATED AND SODIUM-TREATED SOILS

Although the effect of rate of acceleration on moisture equivalent values has been recognized by others as a factor (*21*), experimental data bearing on it have not been found in the literature. The measurements reported in table 3 show higher moisture equivalent values when the drum is brought to full speed in 15 seconds than when full speed is attained in 4.5 minutes. The effect is especially marked only in the instance of Na soils 7 and 10. Relatively slower accelerations have been most extensively used in the past; otherwise a rapid accel-

eration with the resulting higher values would seem to be desirable, since a closer packing of soil particles is more representative of field conditions.

TABLE 3.—*Effect of starting acceleration on the moisture equivalent of Ca and Na soil*

Time to full speed	Moisture equivalent of soil receiving indicated treatment							
	Soil No. 6		Soil No. 10		Soil No. 5		Soil No. 7	
	Ca	Na	Ca	Na	Ca	Na	Ca	Na
15 seconds	13.2	23.2	21.5	43.0	22.0	37.5	22.2	57.6
4.5 minutes	12.8	21.8	20.7	32.4	20.5	35.6	21.2	51.6

MIGRATION AND SEGREGATION OF SAND, SILT, AND CLAY IN DISPERSED SOILS DURING CENTRIFUGING

Two characteristics of certain of the centrifuged Na soils attracted attention at the outset of this investigation. At the end of the 30 minutes in the centrifuge, free water was present on the surface of the finer-textured Na soils 3, 5, 7, 8, 9, and 10. After these soils were dried the surfaces were glazed, and many times an upper layer curled away from the underlying soil (fig. 1), indicating a segregation of fine particles.

Examinations indicated that the upper layer was composed almost wholly of clay particles. Beneath this clay, silt particles tended to predominate. In soil 7, there was a clearly demarked light-colored silt surface and sand particles were observed to have accumulated in the outer soil near the filter paper. These effects will receive further consideration in the section that follows.

DISTRIBUTION OF MOISTURE IN CALCIUM-TREATED AND SODIUM-TREATED SOILS AFTER CENTRIFUGING

Measurements were made of the distribution of water after centrifuging in five of the Na soils and, for comparison, in the corresponding Ca soils by the following procedure. The original screens were removed from a number of the centrifuge cups and others substituted that could be slipped into place and readily removed. The soils were prepared for the moisture-equivalent determination in the usual way, by weighing 30 gm. of air-dry soil into the cups, allowing them to stand for 24 hours in a tray of water, and gently adding water to the surface of any that had not taken up enough water to wet the surface within half an hour. After the soils had drained for 30 minutes, the cups were placed in the centrifuge drum without jarring and centrifuged for 30 minutes. The cups were taken from the drum, and if there was free water on the surface they were inverted for a few minutes and the inside walls of the cups wiped with absorbent paper. The screens were then carefully slipped off the bottom of the cups and the soils placed on a block of wood slightly concave along the center line and a little smaller than the inside of the cup. By carefully pulling the cup downward, it was possible to expose portions of soil of the desired thickness above the upper edges of the cups. Successive layers, roughly 2 mm. thick, were in turn sliced off with a knife and transferred to weighing cans. Four layers were taken from

each of the Na soils and three from each of the Ca soils. The bottom section of the Ca soils exceeded 2 mm. in thickness, but there was not sufficient for a full 2-mm. fourth layer. These methods, though not very exact with respect to the thickness of the soil layers, were regarded

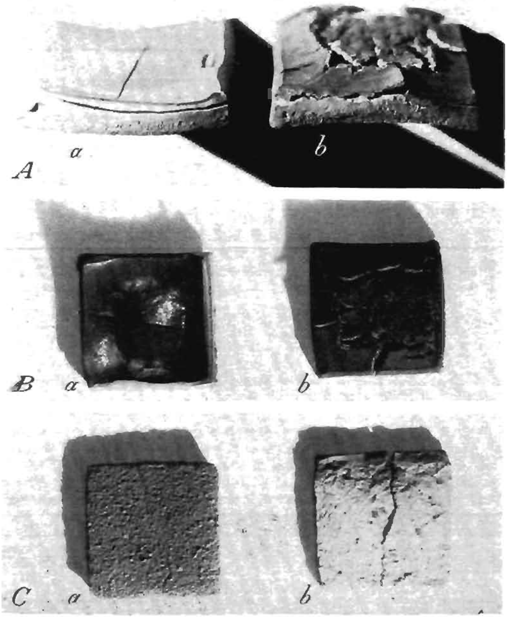

FIGURE 1.—Appearance of centrifuged Ca- and Na-treated soils after drying: *A* and *B*, (*a*) Na soil 10 and (*b*) Na soil 7 after centrifuging and drying; *C*, (*a*) Ca soil 10, (*b*) Na soil 7 with clay layer removed, exposing white calcareous silt layer.

as nonetheless suited to the purpose. Difficulty from crumbling, such as was experienced with Ca soil 5, would have been encountered had the measurements been undertaken with the coarser-textured Na soils.

In conformity with the findings of Veihmeyer, Israelsen, and Conrad (*22*), who worked with untreated soils, it was found (table 4)

that the percentage of water in the Ca soils increased from the inner surface to the outer. The water distribution in each of the Na soils stands in contrast. With these the gradient was reversed. In three of the five Na soils over twice as much water was present in the inner layer as in the outer.

TABLE 4.—*Effect of sodium on distribution of moisture in centrifuged samples*

Soil No.	Moisture content of successive indicated layers of soil from inner surface outward toward periphery of centrifuge in—								
	Ca soils				Na soils				
	0–2 millimeters	2–4 millimeters	Last layer	Weighted mean	0–2 millimeters	2–4 millimeters	4–6 millimeters	Last layer	Weighted mean
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
					48.4	34.4	29.6	27.8	35.1
5	(¹)	(¹)	(¹)	-------	53.8	38.4	32.1	28.4	36.2
					49.0	39.0	34.2	29.9	35.5
					99.5	64.3	48.0	40.4	55.0
7	19.8	21.5	22.7	20.8	94.4	55.7	50.9	44.2	55.5
					76.0	57.5	55.0	47.9	57.8
8	21.5	22.1	23.2	22.2	52.3	39.1	34.3	31.6	38.2
	21.2	22.1	22.2	21.8	52.1	39.1	34.3	31.5	38.1
9	16.3	16.4	17.1	16.6	63.3	24.9	22.2	22.0	30.8
	16.8	17.0	17.3	17.0	-------	-------	-------	-------	-------
10	20.0	20.2	20.5	20.2	78.4	33.9	29.5	27.2	38.3
	20.7	20.1	20.9	20.5	-------	-------	-------	-------	-------

¹ Crumbled.

In considering these effects it is necessary to take into account one of the important characteristics of the moisture-equivalent determination. The centrifugal force of 1,000 times gravity is applied not alone to the water, but also to the soil particles. As placed in the centrifugal drum, Na soils contain much water and being dispersed an excellent condition is provided in the plastic masses of the fine-textured soils for particle segregation. The large particles of Na soils, in accordance with Stokes' law, tend to migrate toward the periphery, displacing small particles toward the axis of rotation. The mass of the finer-textured Na soils being somewhat impermeable, a part of the water, which has a specific gravity below that of hydrated clays, is displaced toward the axis of rotation and collects on top of the clay. Accompanying this inward displacement of water there is a further sorting of clay and silt particles by size and specific gravity, giving rise to segregations such as are illustrated in figure 1. Continued centrifuging of Na soils beyond the 30-minute period resulted in some further loss of water, but the rate of movement of water out of the soil was so slow as to eliminate the possibility of removing surface water by any reasonable period of centrifuging.

EFFECT OF ADSORBED SODIUM ON THE MOISTURE EQUIVALENT OF THE 12 SOILS

The moisture equivalents of each of the 12 Ca and Na soils are shown in table 5. In this table there are also shown the clay content and exchange capacity of these soils and the adsorbed sodium of the Na soils.

The moisture equivalents of soils 279, Gold Ridge sandy loam, and 278, Aiken clay, were decreased by treatment with sodium. Soil 279

is highly siliceous, contains little clay, and has a low exchange capacity. Soil 278 is of basic igneous origin, and although it has an apparent clay content of 37.4 percent it possesses few clay properties. The volume weight of this soil is only 1.09, and the ultimate soil particles are irregular in shape, porous, and easily broken. It seems probable that the indicated high clay content may be more apparent than real, since in the mechanical analysis the settling rates of many of the larger fine particles would be slow enough to cause them to be drawn off as clay. Anderson (*1*) found the moisture equivalent of the colloidal fraction of a lateritic soil to be unaffected by sodium treatment.

TABLE 5.—*Effect of adsorbed sodium on the moisture equivalent*

Soil No.	Moisture equivalent			Clay less than 0.002 mm.	Exchange capacity per 100 gm.	Adsorbed sodium per 100 gm.	
	Ca soil	Na soil	Increase in Na soil over Ca soil				
				Percent	*Milliequivalents*	*Milliequivalents*	*Percent of total*
279	10.2	9.4	----------	6.5	3.34	2.44	73.1
1	9.2	10.2	1.0	5.9	4.82	3.04	62.7
4	11.2	15.5	4.3	9.2	7.13	3.96	55.5
2	11.9	16.6	4.7	9.6	8.54	4.44	52.0
6	13.3	23.2	9.9	9.1	8.02	4.88	60.8
3	14.5	[1] 33.0	18.5	12.0	9.32	6.16	66.1
9	18.6	[1] 34.0	15.4	25.7	14.05	8.87	63.1
5	21.9	[1] 37.5	15.6	18.4	16.46	8.17	49.6
10	21.4	[1] 42.9	21.5	30.1	15.83	12.26	77.4
8	22.6	[1] 43.7	21.1	21.0	14.82	7.79	52.6
7	22.1	[1] 57.4	35.3	27.3	20.00	12.75	63.8
278	33.0	30.1	----------	26.6	14.77	2.31	15.6

[1] Free water on soil after centrifuging.

The soils are listed (278 excepted) in the order of increasing Na soil moisture equivalents (table 5). Tending to parallel this order, there are increases in the difference between the Ca soil and Na soil moisture equivalents, in clay content, in exchange capacity, and in adsorbed sodium. A closer parallelism would scarcely be expected when account is taken of the variations in the size of clay particles here grouped as less than 0.002 mm., in the hydration characteristics and exchange capacity of different clays, and the differences in soils with respect to particle segregations in the centrifuge. The conclusion obviously follows that the moisture equivalent of soils reflects not only mechanical composition as determined by standard analysis but also soil structure as influenced by the kind and quantity of the adsorbed cations.

The effect of adsorbed sodium on the moisture equivalent is regarded as being consequent to three coincident factors: Hydration, dispersion, and the segregation of a relatively impervious layer of clay on the surfaces of some of the soils. Hydration contributed to the results both because of the tightly held water and because of such relation as hydration may bear to impermeability. Dispersion increased the free surfaces and thereby the water retentiveness of the soils.

THE MOISTURE EQUIVALENTS OF CALCIUM-TREATED SOIL AND SODIUM-TREATI SOIL MIXTURES

When the Ca and Na soils were mixed in equal proportions by pr longed rolling in a sheet of paper, the resulting moisture-equivale values tended to approach in magnitude the Ca soil values more nearl than the Na soil values (table 6).

TABLE 6.—*The moisture equivalent of mixtures of calcium- and sodium-treated so*

Soil No.	Moisture equivalent of indicated percentage mixture of Ca soil and Na soil [1]										
	100/0	90/10	80/20	70/30	60/40	50/50	40/60	30/70	20/80	10/90	0/10
279	10.2					10.0					9
1	9.2					10.1					10
4	11.2					13.0					15
2	11.9					12.7					16
6	13.3	13.2	13.8	13.3	14.6	15.1	15.6	17.7	18.8	20.4	23
3	11.5					17.2					[2] 33
9	18.6					22.2					34.
5	21.9	21.3	21.8	22.5	22.5	23.4	25.2	26.9	30.2	[2] 36.2	[2] 37
10	21.4	21.6	21.7	22.3	24.5	[2] 27.2	[2] 30.5	[2] 33.3	[2] 37.7	[2] 41.3	[2] 42
8	22.6					27.0					[2] 43
7	22.1	21.9	22.8	23.9	27.0	30.6	[2] 38.4	[2] 41.0	[2] 45.5	[2] 54.8	[2] 57
278	33.0					31.6					30

[1] Numerator=Ca soil; denominator=Na soil.
[2] Free water on soil after centrifuging.

The fact that sigmoid moisture equivalent curves result when C and Na soils are mixed in the successive proportions shown in figur 2 would indicate that the relation between adsorbed sodium and eithe or both hydration and dispersion is not a linear one. Some of Ratner' data (*13*) on the dispersion of clay suspensions by adsorbed sodiun also yield a graph with slightly sigmoid characteristics. Bodman an Mahmud (*2*) found a straight line relation between the moistur equivalents of successive mixtures of sand and clay. The migratio and accumulation of clay and water on the surfaces of the soils show in figure 1 unquestionably influenced the shape of the curves. In th soil mixtures where calcium greatly exceed sodium there was littl dispersion and, without dispersion little migration. The fact tha the upper portions of the curves of soils 7, 10, and 5 are flattened i due in part to the free water. The quantity of free water was observed to increase with the proportion of the Na clay in the mixture. The upper part of the curve soil 6 is not flattened and this soil had no free water on the surface of any of the samples.

The first evidence of a significant increase in the moisture equivalent of soil 6 occurred in the 60/40 mixture, corresponding to 1.9 milliequivalents of adsorbed sodium per 100 gm. of soil. The moisture equivalents of soils 5 and 10 appear to have been increased slightly by the substitution of 30 percent of Na soil, corresponding respectively to 2.4 and 3.6 milliequivalents of adsorbed sodium. The moisture equivalent of soil 7 was increased by the substitution of 20 percent of the Na soil, corresponding to 2.5 milliequivalents of adsorbed sodium. It seems from these limited data that the moisture equivalents of soils are not measurably affected by less than 2 milliequivalents of adsorbed sodium.

The maximum effect on the moisture equivalent was approached in soils 5, 10, and 7 with, respectively, 8.17, 12.26, and 12.75 milliequivalents of adsorbed sodium, corresponding to 49.6, 77.4, and 63.8

percent of sodium in the exchange complex. The maximum effect on the moisture equivalent of soil 6 was not reached or apparently approached with the 4.9 milliequivalent of adsorbed sodium in the 0/100 mixture.

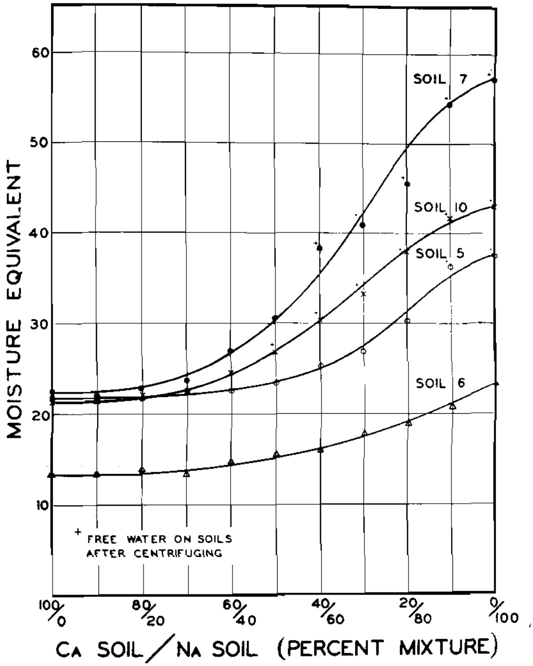

FIGURE 2.—The moisture equivalents of various mixtures of different soils that had been treated with calcium and sodium.

THE MOISTURE EQUIVALENT OF SODIUM-TREATED SOILS WET WITH STRONG ELECTROLYTES

Soil colloids, irrespective of the character of the adsorbed bases, are flocculated when suspended in solutions of strong electrolytes. It follows that adsorbed sodium should be without effect on the moisture equivalent when samples are wet with a strong salt solution rather than with distilled water. The results of measurements made on each of the 12 sodium-treated soils as wet with normal calcium

chloride, and where sufficient soil was available, with normal sodiu chloride, are reported in table 7. It is to be observed that th moisture equivalents of the Na soils wet with these solutions tende to be equal to, or slightly lower than, the moisture equivalents (Ca soils wet with distilled water.

TABLE 7.—*Moisture equivalents of Na soils wet with normal $CaCl_2$ and NaCl solutio*

Soil No.	Moisture equivalent of—			
	Na soil wet with normal—		Soil wet with distilled water in usual manne	
	$CaCl_2$	NaCl	Ca soil	Na soil
279	10.2	9.4	10.2	9.
1	10.0	9.8	9.2	10.
4	11.6	10.2	11.2	15.
2	10.9	12.0	11.9	16.
6	13.6	13.1	13.3	23.
3	14.2	14.2	14.5	33.
9	18.5	18.2	18.6	34.
5	[1] 20.7		21.9	37.
10	21.4	21.1	21.4	42.
8	22.9	22.6	22.6	43.
7	20.9		22.1	57.
278	29.7	29.6	33.0	30.
Average	17.0		17.5	29.

[1] Determined on two 10-gm. samples (values, 26.6 and 25.9 percent) and interpolated after Veihmeye Israelsen, and Conrad (*22, table 1, fig. 1*) to 30-gm.-basis.

It is suggested by these results that the moisture equivalent of fiel soils if determined comparatively (1) as wet with distilled water an (2) as wet with normal $CaCl_2$ or NaCl should provide an index to sodium-induced dispersion and hydration and by inference to reduce permeability from these causes. If saline, the moisture equivalent of Na and Ca soils as taken from the field should be similar. A thir and companion measurement is accordingly necessary for a practica utilization of the moisture equivalent as an index to adsorbed sodium namely, the moisture equivalent of the soil determined after most o the readily soluble electrolytes have been removed by washing and extraction with filter candles. The moisture equivalent so used should provide a practical index, both to the presence of substantia amounts of adsorbed sodium in field soils and to the consequent changes in the permeability characteristics that would be expected to accompany reclamation with nonsaline waters.

The similarity of the moisture equivalents of Ca and Na soils wet with strong electrolytes would seem to eliminate the possibility that the effect of sodium on the moisture equivalent of soils should be attributed to puddling or the mechanics of handling prior to the measurements.

An observable redistribution of soil separates did not occur when the Na soils were wet with strong electrolytes, indicating that the electrolytes increased the attractive forces between particles sufficiently to check migration and the displacement of light particles toward the axis of rotation. The surfaces of the Na samples wet with either of the normal salt solutions resembled the surfaces of the Ca samples, and in no case was free water present on the surface after centrifuging.

The laboratory measurement of the permeability of soils, even on a elative basis, has proved to be a difficult problem in many hands, ut some promise of progress is afforded by other measurements wherein soils were leached with solutions of the same composition as heir own displaced solutions. The use of such solutions minimizes ase exchange and the associated effects on dispersion during suc-essive leachings. By using this procedure creditable agreements ave resulted between quadruplicate samples, and the hour-to-hour hanges in rate of percolation so often characterizing other per-neability measurements have been greatly reduced.

FFECTS OF CALCIUM, MAGNESIUM, POTASSIUM, AND SODIUM ON THE MOISTURE EQUIVALENT AND HYGROSCOPICITY

The comparative effects of Ca, Mg, K, and Na on the moisture quivalent of soils have been studied only by Anderson (*1*), and he onfined his work to the colloidal fraction. It seemed highly probable hat Anderson's results might be applied to soils in general, but the ignificance of the relationships was regarded as sufficiently important o justify independent measurements. The question involved that ies in most closely with irrigation considerations was whether there vas ample reason for regarding calcium and magnesium as alike in heir physical effects on soils and, if so, whether such potassium as is ound in irrigation waters should be classed with the calcium and nagnesium or included with sodium in the calculation of the sodium-otal base ratio (percent sodium).

The treatments employed in the preparation of the soils used for hese experiments, as described earlier in this paper, were more drastic han those used in preparing the Ca and Na soils. Extensive prediges-ions were made with ammonium acetate and ammonium hydroxide, which were designed to reduce all soils to a common ammonium base nd to remove calcium carbonate and humus, but these digestions vere not carried to the ultimate end points. Calcium was found in the inal ammonium acetate extractions of all soils and humus appeared n some of the first washings of the K and Na soils. Presumably all f these soils approached complete saturation with the respective ases, but no measurements were made. Distilled water washing was ontinued until the chloride concentrations of the final suspensions vere less than 5 milliequivalents per liter.

The moisture equivalents of these soils are compared in two ways, *1*) when wet with distilled water and (2) when wet with 0.02 normal hloride solutions of the bases corresponding to those adsorbed. The verages of triplicate moisture equivalent measurements are reported xcept in occasional instances when one of the three values was out of ine with the other two.

The moisture equivalents of Ca and Mg soils are found to be similar table 8) and nearly always less than the K soil. The effect of the odium ion in this experiment, as in the preceding one, is outstanding, number of the Na soils yielding moisture equivalent values twice s great as the Ca soils. This is essentially the effect which Anderson *1*) obtained, but in his series, as in this one, there was one colloid, in ddition to the laterite, upon which sodium had little or no effect.

In the presence of 0.02 normal electrolyte, the moisture equivalents f the Ca and K soils were reduced a little, but a consistent effect is ot shown in the Mg series. The effect of the 0.02 electrolyte on the

Na soils is marked, but the values are nearer to the Na soil values than to the Ca soil values. From the standpoint of considerations related to the effects of irrigation waters on soils, it seems appropriate to group calcium, magnesium, and potassium ions together and to differentiate these from the sodium ion.

TABLE 8.—*Moisture equivalents of Ca, Mg, K, and Na soils wet with distilled water and 0.02 normal electrolyte*

Soil No.	Moisture equivalent of soil—							
	Wet with water				Wet with 0.02 normal electrolyte			
	Ca soil	Mg soil	K soil	Na soil	Ca soil	Mg soil	K soil	Na soil
279	9.7	10.6	9.7	10.5	9.7	10.7	9.2	10.[illegible]
1	9.4	9.8	10.1	10.6	8.9	9.3	9.2	10.8
4	11.7	11.0	11.1	15.7	11.3	11.3	10.3	15.4
2	10.9	10.5	11.0	15.5	10.6	11.3	10.3	15.2
6	12.7	12.9	13.0	18.8	12.2	12.8	11.8	16.4
3	14.0	14.2	15.2	23.6	13.2	14.2	13.9	20.6
9	19.4	19.9	20.1	[1] 33.7	19.1	20.1	18.5	[1] 30.7
5	20.8	22.0	23.7	[1] 45.0	21.1	21.4	22.8	30.4
10	23.0	25.0	26.6	[1] 52.3	23.9	22.6	24.8	[1] 47.4
8	23.3	23.8	26.6	[1] 62.5	22.6	23.8	25.2	39.5
7	22.9	23.6	24.4	[1] 85.3	21.8	23.3	22.6	[1] 54.8
278	36.1	36.1	34.6	32.4	35.2	35.2	32.7	32.6
Average	17.9	18.1	18.8	33.8	17.5	18.0	17.6	27.0
Average effect of electrolyte					−.4	−.1	−1.2	−6.8

[1] Free water on soil after centrifuging.

The influence of the kind of adsorbed base on the hygroscopicity of soils was investigated as an incidental feature of the inquiry by the following procedure. The three oven-dried moisture-equivalent samples of each of the soils of the experiment reported in table 8 were ground and mixed. From each of these, two 25-gm. samples were weighed out and placed on 4-inch watch glasses and exposed on open shelves in a closed concrete basement vault for 15 days. The atmosphere in this vault was humidified by a fan directed into a group of wet towels suspended from a vessel of water. The same fan maintained a good circulation of air throughout the room and over the soils. The average temperature in this vault, as recorded by a thermograph during the period of the experiment, was 20.3° C. (during the 15-day period the maximum was 21.1° and the minimum 18.8°). The average relative humidity during the first 7 days was about 72 percent and during the last 8 days it was 84.1 percent (lowest 81.0 and highest 90.0) as determined twice daily with a sling psychrometer. The partial pressure of water in an atmosphere of 84 percent relative humidity at 20.3° C. is 1.9 cm. Each soil was stirred daily and left ridged to give as great a surface as possible. Starting on the 12th day, selected samples were weighed twice daily until the 15th day by which time the variations were negligible and the gains equaled the losses The samples were then placed in bottles, and their moisture content was determined by weighing them before and after oven-drying at 105°–110° C. In considering these data, account should be taken of the fact that they are wetting data in contrast with the drying curves represented by the wilting coefficient and ultimate wilting-point results presented in figure 3.

TABLE 9.—*Hygroscopicity of oven-dried Ca, Mg, K, and Na soils when in equilibrium with an atmosphere of 84 percent relative humidity at 20.3° C.*

Soil No.	Hygroscopicity of oven-dried soil—							
	Previously wet with distilled water				Previously wet with 0.02 normal electrolyte			
	Ca soil	Mg soil	K soil	Na soil	Ca soil	Mg soil	K soil	Na soil
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
279	0.93	1.02	0.81	0.87	0.94	1.14	0.93	0.86
1	.90	1.04	.81	.92	.99	1.07	.80	.98
4	1.62	1.65	1.28	1.30	1.65	1.73	1.17	1.54
2	1.75	1.81	1.27	1.46	1.78	1.91	1.35	1.77
6	2.24	2.14	1.63	1.95	2.15	2.23	1.66	1.97
3	2.41	2.31	1.78	2.22	2.34	2.42	1.91	2.24
9	3.75	3.90	2.98	3.15	3.87	4.18	3.02	3.75
5	4.23	4.37	3.15	3.90	4.27	4.52	3.23	3.94
10	4.74	4.70	3.80	4.53	4.98	4.94	3.97	4.85
8	3.94	4.12	3.13	4.20	4.24	4.16	3.20	4.25
7	5.18	5.42	3.81	4.67	5.29	5.62	3.73	4.76
278	7.42	8.06	7.51	7.86	7.50	8.14	7.82	7.57
Average	3.26	3.38	2.66	3.09	3.33	3.51	2.73	3.21
Average effect of electrolyte					+.07	+.13	+.07	+.12

At the end of the absorption period the theoretical concentration of electrolytes in the moisture held by the soils previously treated with 0.02 normal salt solutions is represented by the relation: Moisture equivalent÷hygroscopic moisture × 0.02. Solution concentrations are indicated as high as 232 milliequivalents per liter in Na soil 279 and as low as 82 milliequivalents in Ca soil 7.

The electrolyte increased hygroscopicity in nearly every comparison (table 9), and its average effect, though small, was nearly the same for each of the four bases with which the soils had been treated.

Calcium-treated soils, in keeping with the findings of Anderson (*1*) and with those of Thomas (*19*) at the latter's higher vapor pressures, adsorbed more moisture than did sodium-treated soils. Except for a few determinations (table 9), the magnesium values are slightly higher than the calcium values. Potassium is the low member of the series. Na soils adsorbed less moisture than did Ca or Mg soils but more than K soils. The order both with and without electrolyte is K<Na<Ca<Mg. These results will be referred to again in the final section.

AVAILABILITY OF SOIL MOISTURE IN CALCIUM-TREATED AND SODIUM-TREATED SOILS

The wilting-coefficient and ultimate wilting-point measurements (tables 10 and 11) show that the moisture in Na soils is less available to plants than that in Ca soils, and in nearly all instances the differences are substantial. The differences between the Ca soils and Na soils decrease as the moisture content is reduced from the wilting coefficient to the ultimate wilting point.

The wilting coefficients and ultimate wilting points of soils 278 and 279 were not changed appreciably by the sodium treatments, and these soils, which adsorbed little sodium, showed no positive sodium effects in the moisture-equivalent comparisons. Through the remainder of the series there is a strong positive relation between the effect of sodium in increasing the moisture equivalent and in increasing the

271522—40——9

TABLE 10.—*Effect of exchange sodium on the wilting coefficient and ultimate wilting point of Ca and Na soils*

WILTING COEFFICIENT (PERCENTAGE OF MOISTURE)

Soil No.	Washed soil in can No.—							Ca soil in can No.—							Na soil in can No.—						
	1	2	3	4	5	6	Average	1	2	3	4	5	6	Average	1	2	3	4	5	6	Average
279	3.8	3.6	3.3	3.4	3.5	3.8	3.6	4.0	3.4	3.7	3.4	3.3	3.9	3.6	----	4.3	4.3	3.8	3.9	4.9	4.2
1	3.5	3.5	3.3	3.5	3.8	3.5	3.5	3.6	3.6	3.7	3.7	3.7	3.6	3.7	3.7	5.2	4.0	5.4	4.4	4.5	4.6
4	5.3	5.0	4.9	5.2	5.2	5.0	5.1	4.4	4.5	4.9	5.0	4.6	4.7	4.7	8.2	----	10.2	----	----	7.2	[1] 8.5
2	6.7	5.1	5.1	4.9	4.8	5.2	5.3	5.8	4.8	5.7	5.6	5.1	5.7	5.5	9.5	7.2	8.1	8.1	8.0	10.1	8.5
6	5.5	5.4	5.6	5.8	5.4	5.4	5.5	5.8	5.5	5.6	5.6	5.9	5.6	5.7	7.0	7.0	7.0	9.0	7.6	8.8	7.8
3	6.0	6.2	6.7	6.5	6.1	5.8	6.2	5.7	5.6	6.2	5.7	5.9	5.8	5.8	9.7	8.1	9.0	11.9	9.3	10.0	9.7
9	10.2	10.0	9.8	10.1	10.1	10.0	10.0	10.4	10.2	10.5	9.8	10.3	10.2	10.2	11.4	12.9	12.5	10.7	11.7	11.6	11.8
5	9.3	10.4	10.1	10.1	9.6	9.8	9.9	9.5	9.5	9.5	9.9	10.0	10.1	9.8	13.8	15.6	14.9	13.6	14.7	15.1	14.6
10	11.9	12.1	13.0	12.3	12.3	12.3	12.3	11.4	12.8	11.7	11.8	12.6	11.5	12.0	14.7	15.8	16.2	16.6	15.9	14.1	15.6
8	10.7	10.3	10.3	10.5	9.7	9.8	10.2	12.7	11.4	10.7	12.1	12.0	11.0	11.7	13.9	15.1	13.7	13.9	12.8	12.6	13.7
7	11.2	11.7	11.2	12.2	12.1	10.9	11.6	11.4	11.2	11.2	11.1	11.9	11.5	11.4	15.9	16.2	15.8	15.8	15.4	15.6	15.8
278	22.2	21.4	22.3	21.5	----	----	21.9	21.2	21.6	22.7	22.3	----	----	22.0	21.8	21.8	22.9	22.9	----	----	22.4
Average	----	----	----	----	----	----	8.8	----	----	----	----	----	----	8.8	----	----	----	----	----	----	11.4

ULTIMATE WILTING POINT (PERCENTAGE OF MOISTURE)

Soil No.	Washed soil in can No.—							Ca soil in can No.—							Na soil in can No.—						
	1	2	3	4	5	6	Average	1	2	3	4	5	6	Average	1	2	3	4	5	6	Average
279	3.4	3.2	3.0	3.0	3.3	3.2	3.2	3.3	3.0	3.1	2.8	2.9	3.4	3.1	----	3.2	3.2	3.3	3.6	3.4	3.3
1	3.3	3.3	3.1	3.3	3.3	3.5	3.3	3.6	3.5	3.6	3.4	3.6	3.3	3.5	3.6	3.9	4.3	4.2	3.8	4.1	4.0
4	4.6	4.6	4.8	5.0	4.6	4.4	4.7	4.4	4.5	4.6	4.4	4.4	4.7	4.5	5.2	----	5.6	----	----	5.2	[1] 5.3
2	4.9	5.0	4.6	4.8	4.7	4.7	4.8	4.6	4.8	4.8	4.9	4.9	4.8	4.8	6.0	5.9	5.8	6.3	6.6	5.7	6.1
6	5.1	5.2	5.0	5.4	5.1	5.3	5.2	5.3	5.2	5.1	5.1	5.0	5.4	5.2	6.8	6.9	7.4	6.6	6.4	6.5	6.8
3	5.5	6.1	5.6	6.0	6.0	5.6	5.8	5.4	5.6	5.7	5.5	5.6	5.5	5.5	6.6	6.5	7.4	7.2	6.9	7.5	7.0
9	9.2	9.1	9.1	10.0	10.0	10.0	9.6	10.0	10.0	9.7	9.0	10.2	9.7	9.8	11.2	10.4	9.6	9.9	10.6	10.3	10.3
5	8.8	9.8	9.3	9.0	8.9	9.2	9.2	9.0	9.5	8.7	9.2	9.0	9.5	9.2	10.2	11.2	10.1	9.9	12.1	11.7	10.9
10	11.4	11.2	11.6	11.5	11.5	12.0	11.5	10.9	10.8	11.2	11.3	11.1	11.0	11.1	12.9	12.5	13.8	12.8	11.8	12.6	12.7
8	10.0	9.6	10.3	10.1	9.2	9.5	9.8	9.8	10.8	10.3	10.9	9.7	11.0	10.4	11.2	12.8	11.0	12.2	12.1	11.4	11.8
7	10.8	10.8	11.2	11.0	10.8	10.7	10.9	11.1	11.1	10.7	10.7	10.9	11.3	11.0	13.2	14.4	14.1	13.0	12.9	13.3	13.5
278	21.3	20.5	21.2	20.8	----	----	21.0	20.6	21.0	22.1	20.9	----	----	21.2	21.1	21.3	22.0	21.6	----	----	21.5
Average	----	----	----	----	----	----	8.3	----	----	----	----	----	----	8.3	----	----	----	----	----	----	9.4

[1] 3 plants in Na soil 4 died before wilting and the remaining 3 were in poor condition.

vilting coefficient and the ultimate wilting point. The coefficient of orrelation between differences at the moisture equivalent and ultinate wilting points of Ca and Na soils for soils numbered 1 to 10 is).76±0.09, which can be regarded as an indication of the operation of at least some of the same causal factors in the two moisture zones.

TABLE 11.—*Summary of moisture equivalents, wilting coefficients, and ultimate wilting points of washed, Ca, and Na soils*

Soil No.	Moisture equivalent			Wilting coefficient			Ultimate wilting point		
	Washed soil	Ca soil	Na soil	Washed soil	Ca soil	Na soil	Washed soil	Ca soil	Na soil
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
79	10.1	10.2	9.4	3.6	3.6	4.2	3.2	3.1	3.3
	10.1	9.2	10.2	3.5	3.7	4.6	3.3	3.5	4.0
	11.2	11.2	15.5	5.1	4.7	[1] 8.5	4.7	4.5	[1] 5.3
	11.5	11.9	16.6	5.3	5.5	8.5	4.8	4.8	6.1
	13.1	13.3	23.2	5.5	5.7	7.8	5.2	5.2	6.8
	13.9	14.5	33.0	6.2	5.8	9.7	5.8	5.5	7.0
	18.1	18.6	34.0	10.0	10.2	11.8	9.6	9.8	10.3
	21.2	21.9	37.5	9.9	9.8	14.6	9.2	9.2	10.9
0	22.2	21.4	42.9	12.3	12.0	15.6	11.5	11.1	12.7
	23.3	22.0	43.7	10.2	11.7	13.7	9.8	10.4	11.8
	23.9	22.1	57.4	11.6	11.4	15.8	10.9	11.0	13.5
78	32.9	33.0	30.1	21.9	22.0	22.4	21.0	21.2	21.5
Average	17.6	17.5	29.5	8.8	8.8	11.4	8.3	8.3	9.4
Average of soils 1 to 10, omitting 4	17.5	17.3	33.2	8.3	8.4	11.3	7.8	7.8	9.2

[1] 3 of the 6 original plants of Na soil 4 died before wilting and the remaining 3 on which the average is based vere in poor condition.

Before continuing the analysis of the results from the standpoint of he soil, the possibility of differences between the plants grown in Ca oils and those grown in Na soils that might affect their ability to itilize soil moisture should be canvassed. McGeorge and Breazeale *12*) have considered oxygen deficiency as a factor responsible for the vilting of plants in puddled soils. Some support for the idea that puddling, as ordinarily considered, was not an important factor contributing to the results of the present work is provided by the facts: 1) That whether measured in the dry state, in the moisture equivalent ups after centrifuging, or in the wilting coefficient cans, the volumes of the Na soils were greater than the volumes of the Ca soils; and (2) hat when the Na soils were wet with strong electrolytes, the moisture equivalent values were nearly the same as those of the Ca soils.

The distribution and the abundance of roots were examined with onsiderable care both in representative cans before oven-drying and fterwards as the successive cans were emptied for grinding and mixng. Differences were not found, but no examinations were made of he comparative abundance of root hairs. If there had been fewer oots, or a poorer distribution of roots in the Na soils than in the Ca oils, wilting would be expected to have occurred at a higher moisture ontent in the Na soils.

It may be assumed that the solutions of the Na soils contained subtantially higher concentrations of sodium ion than did the solutions of the Ca soils. It is accordingly possible that physiological distinctions should be drawn between the two sets of plants, but so little is nown about sodium effects on tissue structure that discussions cannot

be carried far in this direction. As distinct from the Ca roots grow on Ca soil, roots grown on Na soil may have been more highly hy drated and water uptake by them conceivably could have been im peded, but such an effect has never been demonstrated. In som earlier sand-culture experiments in which plants were grown on nutri ent solutions high in calcium chloride and high in sodium chlorid respectively, the plant material from the sodium solutions when place on a Büchner funnel, after drying and grinding, took up and hel relatively large quantities of water and could be leached only wit difficulty, whereas the calcium plant material behaved in a normal way The question of whether sodium affects the uptake and movement o water in living plants must be regarded as speculative and there is n present basis for a conclusion.

CAPILLARY POTENTIAL

The term "availability" as phrased by Richards (*14*) involves tw notions, namely, (1) the ability of the plant to absorb and to use wate with which it is in contact and (2) the readiness or velocity (*6*, *14* with which the soil moisture moves in to replace that which has bee used by the plant. At any given point in the soil water, the capillar potential is numerically equal to the hydrostatic potential and belov saturation the quantity is negative. The tension of the soil water a a given capillary potential may be expressed as the length of th suspended water column necessary to produce that tension. Th rates of water movement through a soil for a given potential differenc will not be the same in different soils, in the same soil at differen capillary potentials, or in the same soils as here differentially treate with calcium and sodium. Furthermore, the moisture at the wiltin coefficient cannot be regarded either as being uniformly distribute throughout the soil mass or as being in static equilibrium with th plant at wilting. In the wilting range, the plant-soil system is a dynamic one and water withdrawal is continuous, though at a decreas ing rate, to the death point.

The present experiments were so conducted as to yield values fo each Ca and Na soil at each of three moisture levels, and to each o these moisture levels it is possible to assign at least an approximat capillary-potential value. Schofield and Botelho daCosta (*16*), fron freezing-point-depression measurements of their own and calculation from vapor pressures and seed-absorption measurements of others have computed the pF value of soils (the logarithm of the equivalen capillary tension expressed in centimeters of water column) at th moisture equivalent and at the wilting coefficient. These value ranged from 2.5 to 3.3 for the moisture equivalent and from 4.02 to 4.40 for the wilting coefficient. From the foregoing data, the writer elected to assume an intermediate value of 3.0 for the moisture equiva lent and to take Schofield's average value, 4.24, for the pF value a the wilting coefficient. The problem remained of obtaining a suitabl value for the ultimate wilting point. By relating the average chang in moisture content between the wilting coefficient and the ultimat wilting point of the writers' calcium soils to the corresponding averag changes in pF values for like moisture changes in the Schofield and DaCosta Botelho graphs, a pF increment of about 0.16 was indicated If this is added to the pF value of the wilting coefficient, approximatel 4.40 is obtained for the pF value of the ultimate wilting point. Fo

the purposes here served it is not necessary to assume that pF 3.0 represents the best moisture equivalent value for any soil or for the writers' soils as differentially treated with calcium and sodium. The moisture content values of certain Na soils that have been centrifuged by standard moisture equivalent procedures are not representative of the moisture tension conditions corresponding to a pF value of 3. If other pF values had been used for the moisture equivalent, the wilting coefficient, or the ultimate wilting point, the slopes of the curves (fig. 3) would have been altered but not their general characteristics.

Figure 3 shows moisture percentage (dry basis) plotted against pF for Ca and Na soils: soil 6, a sandy loam; soil 7, a clay; and the aver-

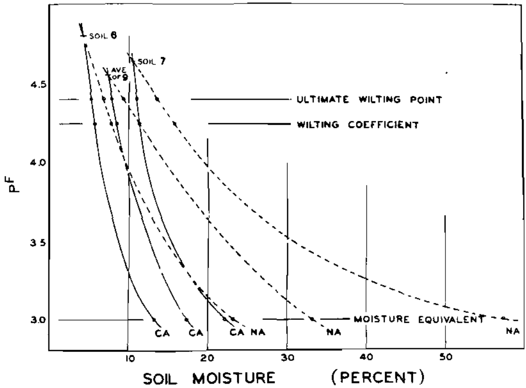

FIGURE 3.—Soil-moisture and pF values of different soils that had been treated with calcium and sodium.

age of the nine soils that showed sodium effects on the moisture equivalent, excluding soil 4, the wilting data for which were not regarded as very accurate. The Ca and Na graphs of corresponding soils are widely separated at the moisture equivalent, but approach each other as the moisture content is decreased, and intersect at a moisture content somewhat lower than the permanent wilting percentage.

From the fact that the Ca and Na graphs intersect, it follows that Ca soils should have higher hygroscopic coefficients than the Na soils in atmospheres substantially below saturation. This finding is in accord with the writers' results (table 9) and those of others.

In keeping with these measurements, it is to be observed that Thomas' (*19*) vapor pressure curves of Ca- and Na-treated soil materials intersected; his Na-treated materials having had a higher moisture-absorbing capacity than his Ca-treated materials from very moist atmospheres and lower from the drier atmospheres. Freezing-point data in a paper by Schofield (*15*) on the penetration of diffuse

double layers may likewise be construed as anticipating the finding of the writers. Those data when extrapolated into the lower moistur range beyond that over which freezing-point measurements ar possible also indicate that water is more closely held by an Na so than by a Ca soil. If extended yet farther the two curves woul intersect.

MOISTURE EQUIVALENT-WILTING COEFFICIENT RATIOS

Conclusions with respect to the effect of sodium on the ratio of mois ture equivalent to wilting coefficient are made difficult by the fac that free water on the surface of many of these soils after centrifugin caused the observed values to exceed what might be regarded as th true values. For Ca soils 1, 2, and 4 these ratios are 2.5, 2.2, and 2.4 respectively, and for the same Na soils 2.2, 2.0, and 1.8. This suggest that sodium tended to narrow the ratios by raising the wilting coeffi cient relatively more than the moisture equivalent.

Treatment with sodium increased neither the moisture equivalent nor the wilting coefficients of soils 279 and 278. These soils are o interest, however, for other reasons. The Gold Ridge sandy loam 279 untreated, has a moisture equivalent-wilting coefficient ratio o 3.0, whereas the Aiken clay has a ratio of 1.6. In the opinion of th writers much of this divergence can be accounted for on the basis o the properties of the ultimate soil particles. The Gold Ridge soil i highly siliceous and the faces of most of the soil particles tend to b smooth, indicating that a large part of the water retained by the soi is exterior to the crystalline surface. A sample of Exeter sandy loan supplied by J. C. Johnston from near Visalia, Calif., had a moistur equivalent nearly four times as great as its wilting coefficient. Th siliceous crystalline character and smooth faces of the particles of thi soil were more outstanding than in the Gold Ridge soil. The par ticles of the lateritic Aiken clay, on the other hand, are highly porou and much water is retained, both against gravitational force anc plant uptake, as closely held interstitial water, i. e., water withir capillary pores. It is obvious that interstitial water retained by a soi both at the moisture equivalent and wilting coefficient would reduc the proportion of moisture-equivalent water available to the plant The interplanar water of a number of soil colloids represents a thirc type of retention, but the question of availability of such water tc plants and its relation to sodium is obviously complex. Much o it is released to the soil atmosphere as the moisture content of the soi is reduced.

DISCUSSION

Whether the consequences of the state of aggregation or dispersior of the ultimate soil particles are more important in terms of watei retention against centrifugal forces and plant uptake than forces involved in the hydration of Ca and Na clays cannot be concludec from these data. Both are probably involved, and the combined soil-water tensions produced are reflected in the water-retention measure-ments. Dispersion may be consequent to hydration; if so, the twc go hand in hand.

The possibility cannot be eliminated that the differences in the availability of moisture in Na soils and Ca soils to plants represent a slower rate of movement through the former soils than through the

latter. In such case it would presumably follow that the soil zones away from the roots were relatively more moist in the Na soils than in the Ca soils, but with equally dry zones in Ca and Na soils at the root surfaces. The distances through which moisture must move to plant roots are relatively great in terms of the thickness of the moisture films at the wilting coefficient. In a soil in which a root or a root hair is situated at intervals of 1 mm., the average distance of water movement is equivalent to 50 times the diameter of a 0.005-mm. silt particle.

If water, in the wilting range, moves primarily by vapor flow, the pertinent distinction between Ca and Na soils in the present connection becomes one of size of capillary pores through which diffusion must take place rather than thickness of water films. Ca soils are characterized by a crumb structure with particle aggregates and voids that are large as compared with those of dispersed Na soils. Vapor diffusion through the Ca soils should accordingly proceed more rapidly than through Na soils.

If moisture movement in the wilting range is primarily movement of film water, it follows likewise that such movement should be slower at a given moisture content in the thinner films covering dispersed particles than in those covering aggregates of closely adhering particles. Water of hydration as such is probably not subject to movement except as it is vaporized into the soil atmosphere.

Irrespective of possible explanations, the finding of immediate significance is that water in dispersed Na soils is less available to plants than that in Ca soils.

SUMMARY

The moisture equivalents of soils partially saturated with sodium were substantially higher than those of the same soils treated with calcium provided most of the soluble electrolyte was removed.

The average moisture equivalents of 12 well-leached Ca, Mg, K, and Na soils were Ca, 17.9; Mg, 18.1; K, 18.8; and Na, 33.8. The average moisture uptake by these soils when exposed (after oven drying) in an atmosphere with a relative humidity of 84 percent at 20.3° C. was K, 2.66; Na, 3.09; Ca, 3.26; and Mg, 3.38 percent

A close parallelism was found for 10 soils between the effect of sodium on the moisture equivalent and the percentage of clay, the exchange capacity, and the quantity of adsorbed sodium.

Adsorbed sodium had negative effects on the moisture equivalent of a soil high in silica and a negative effect on an Aiken clay which is lateritic; both soils had low cation exchange capacities.

The moisture in centrifuged Ca soil samples increased from the inner toward the outer surfaces. An opposite gradient was found in Na soils.

Some migration and segregation of sand, silt, and clay particles occurred when the finer-textured Na soils were centrifuged, a higher proportion of large particles being observed in the outer portions with well-defined clay layers on the inner surface. In certain of the soils water was also displaced toward the axis of rotation and remained on the surface after centrifuging.

Rapid starting acceleration of the centrifuge gave higher moisture-equivalent values than slow acceleration.

Wetting Na soils with normal calcium or sodium chloride solutions gave moisture equivalent values that were nearly the same as those of Ca soils. The moisture equivalent may therefore provide a measure of sodium-induced dispersion of field soils if comparisons are made between leached and unleached soils and soils wetted with a strong electrolyte.

The moisture equivalent of mixtures of Ca and Na soils in successive proportions gave sigmoid graphs. The moisture equivalent was not significantly affected by less than 2 milliequivalents of adsorbed sodium per 100 gm. of soil and values approaching maximum were found when there were 12 milliequivalents or more of adsorbed sodium.

Adsorbed sodium caused soil moisture to be less available to plants. The calcium and sodium averages of nine soils were, at the moisture equivalent, 17.5 and 29.5; at the wilting coefficient, 8.4 and 11.3; and at the ultimate wilting point, 7.8 and 9.2 respectively.

Plotting moisture content against a pF scale, the Ca and Na soil graphs intersect at moisture percentages below the ultimate wilting point. This result confirms earlier work showing lower hygroscopicity of Na soils than of Ca soils in drier atmospheres.

It is suggested that interstitial water held within soil particles against both centrifugal and plant-uptake forces may account for the differences observed in the moisture equivalent-wilting coefficient ratios observed in different soils.

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MODIFICATION OF DIURNAL TRANSPIRATION IN WHEAT BY INFECTIONS OF PUCCINIA TRITICINA [1]

By C. O. Johnston, *associate pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and E. C. Miller, *plant physiologist, Kansas Agricultural Experiment Station* [2]

INTRODUCTION

Earlier investigations (*4*) [3] on the effect of leaf rust (*Puccinia triticina* Eriks.) infection on the water economy of the wheat plant proved that heavy attacks of the disease greatly increased the water requirement of plants of a susceptible variety. It was observed that, while rust-free plants used a greater total amount of water, heavily rusted plants lost more than the controls per unit of dry matter. It also was noted that rusted plants apparently lost water more rapidly in proportion to their surface than rust-free plants. This suggested that leaf rust infection had considerable effect on the transpiration of the host plant and that this factor might lead to severe injury during a shortage of soil moisture. The experiments herein described were designed to measure the transpiration of rusted and rust-free wheat plants and to determine its rhythmic nature.

REVIEW OF LITERATURE

Müller-Thurgau (*7*) studied the effect of various leaf spots on the transpiration of pear, melon, strawberry, and grape, but Blodgett (*1*) was the first to report (1901) on the effect of rust on the transpiration of a host plant. He observed that plants of a species of *Rubus* attacked by *Gymnoconia interstitialis* (Schl.) Lagh. transpired nearly twice as much as rust-free plants. Montemartini (*6*), using cut twigs or shoots bearing rusted leaves placed in water covered with a thin layer of oil, observed that rusts on clematis, violet, rose of sharon, rye, and rose all increased the loss of water by the host plants. He also observed that certain leaf spots increased the loss of water by leaves of several other species of plants. Reed and Cooley (*9*), studying the effect of *Gymnosporangium juniperi-virginianae* Schw. infection on the transpiration of apple leaves concluded that it resulted in lower transpiration. Some diseased leaves were observed to have a water loss only one-fourth as great as comparable healthy leaves.

Apparently the first experiments on the effect of rust infection on transpiration in wheat were conducted by Weaver (*10*), who reported that heavy rust infections greatly accelerated the rate of transpiration. Later, Weiss (*11*) recorded that stem rust infection increased

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[2] The writers gratefully acknowledge indebtedness to H. B. Humphrey, of the Division of Cereal Crops and Diseases, for valuable criticisms and suggestions, and to Hugh G. Gauch, graduate assistant, Kansas Agricultural Experiment Station, for assistance during the course of the experiments.

[3] Italic numbers in parentheses refer to Literature Cited, p. 443.

the transpiration of wheat plants but that the differences were not significant for leaf rust.

Nicolas (*8*), using a method similar to that used by Montemartini, found that several rusts increased the transpiration of the host plants from 1.01 to 2.13 times. He also observed that certain smut diseases increased the transpiration of the host. Kourssanow (*5, pp. 350–353*) first reported the effect of smut on the transpiration of a cultivated cereal crop in 1928. He found that wheat plants attacked by *Ustilago tritici* (Pers.) Rostr. transpired 20 percent more than smutfree plants.

Graf-Marin (*3*) studied the effect of mildew caused by *Erysiphe graminis* f. sp. *hordei* on the transpiration of barley plants. He found that diseased plants transpired 14.62 percent more than healthy plants and that the transpiration per unit area was 66.64 percent higher for diseased than for healthy plants. He found also that diseased plants transpired 15 percent more at night than the disease-free control. In searching for the cause of this phenomenon he discovered that the stomata of diseased plants opened wider and that they opened an hour earlier and closed an hour later than those of the controls. He also found that part of the loss of water by mildewed plants was due to the aerial mycelium of the fungus.

Yarwood (*12*) investigated the effect of powdery mildew caused by *Erysiphe polygoni* DC. on the transpiration of excised leaves of red clover under various conditions. The mildewed leaves were found to transpire about the same as healthy leaves during the day but much more than disease-free leaves at night.

Gassner and Goeze (*2*) investigated the effect of infections of *Puccinia glumarum* (Schm.) Eriks. and Henn. on transpiration and assimilation in wheat. They reported in 1936 that heavy rust infection on a susceptible variety greatly increased transpiration and decreased assimilation. The increase was thought to be due to the combined transpiration of the leaf blades and the fungus.

METHODS AND MATERIALS

The experiments were conducted in the greenhouse during the years 1934, 1935, and 1936. Wheat (*Triticum aestivum* L., syn. *T. vulgare* Vill.) plants were grown to maturity in sealed 1-gallon stone jars, each provided with the watering arrangement described in an earlier paper (*4*). A sufficient quantity of good quality garden loam was screened and mixed with a small quantity of fine sand, and the mass was brought to 45 percent of its water-holding capacity. The soil mass was mixed thoroughly and allowed to stand overnight, after which it was again stirred before being used to fill the jars. To facilitate weighing, all jars were brought to equal weight with coarse gravel. The soil was lightly tamped in each jar to render it firm but not hard. It was added a little at a time until there was the same weight of soil in each jar. As soon as the jars had been filled they were sealed with a thin layer of hot paraffin. Five openings were then cut through the seal at equally spaced intervals about 1 inch from the edge of the jar. A clean, well-formed kernel of wheat was pressed into the soil at each opening, and a small quantity of water was added to expedite germination. The jars were then weighed and each was kept at its respective original weight until all seedlings had emerged. At the end of 2 or

3 days, the few seeds that had not germinated were replaced by fresh kernels.

When perfect stands of five seedlings per jar were obtained, all jars were brought to equal weight by the addition of leached sand to the top of the paraffin seal. This effectively sealed the small openings around the plants, any small breaks in the paraffin seal, and the small space that usually developed after a few days between the edge of the paraffin and the jar. Blank control jars set up in this manner and maintained without plants throughout the season proved the method of sealing to be effective.

During the growth of the plants the moisture content of the soil was maintained at approximately the same point by bringing the jars back to their original weight at frequent intervals. During the early stages of growth, watering every second day was found to be sufficient, but later the jars were brought back to constant weight every day. Graf-Marin (*3*) used automatic soil irrigators, stating that daily weighings allowed too much variation in soil moisture, but the writers found the latter method satisfactory.

Two varieties of white spring wheat of Indian origin were used in the experiments of 1934 and 1935, because of their early maturity and strong straw. One of these, Pusa No. 4 (C. I.[4] 8899), is an awnless variety, very susceptible to the physiologic race of leaf rust that was used for inoculum. The other, an awned unnamed hybrid selection of Pusa 52 × Federation (C. I. 11764), is moderately resistant to the same race of rust in the seedling stage and highly resistant in the adult stage. In order to eliminate any differences in the water relations due to the awns of this selection, the hard red spring variety Reward (C. I. 8182) was substituted for it in 1936. This is an awnless selection, having a moderate type of resistance to leaf rust similar to that of Pusa 52 × Federation. It was found to have only slightly more uredia of leaf rust in the heading stage of growth than the unnamed hybrid.

When the plants had reached the desired stages of growth, some of each variety were placed in a large moist chamber, inoculated with a pure culture of leaf rust physiologic race 9, and after 24 hours removed to the greenhouse bench. Inoculation was accomplished by shaking heavily rusted seedlings above the moistened leaves. The controls were moistened and placed in the moist chamber for 24 hours but were not inoculated.

Excellent infection developed on susceptible plants, and much flecking accompanied by a sparse development of uredia appeared on plants of the resistant variety. In the latter there was a moderate development of uredia on the primary leaves in the seedling stage of growth, but at heading time there was only a sparse development of small uredia, accompanied by much flecking. The uninoculated control plants remained rust-free throughout all the experiments, even though they occupied space within a few feet of heavily infected plants.

Rust readings were made on all infected plants when the rust reached full development. Readings were made on each leaf in terms of percentage in conformity with the rust scale used by the Division of Cereal Crops and Diseases, United States Department of Agri-

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

culture. From the many readings obtained, the average percentag infection for each experimental group was determined.

As soon as rust infection was fully developed on the leaves of inocu lated plants, all jars were brought to constant weight and were weighe thereafter at regular intervals for a period of several hours to de termine the rate of loss of water through transpiration. In 1934 weighings were made every hour, but it was found that changes i weight were more consistent when the weighings were made at 2-hou

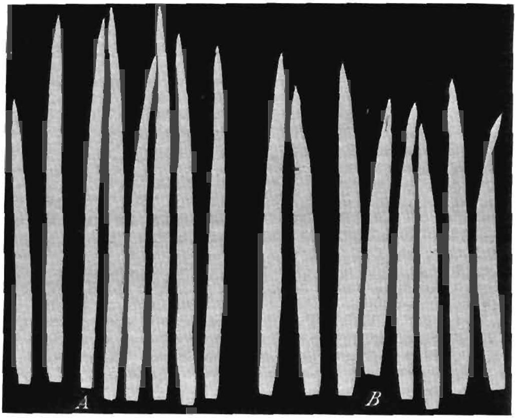

FIGURE 1.—Photograph of blueprint showing differences in size and shape of flag leaves of (A) Pusa No. 4 and (B) Pusa 52 × Federation spring wheat.

intervals. The latter method was used in 1935 and 1936. With all the jars the weighings were started in the morning and continued throughout the day. The experiments of 1934 were run from 8 a. m. to 9 p. m. on each of 2 consecutive days. In 1935, weighings were made from 8 a. m. to midnight on each of 2 consecutive days, and in 1936 readings were made at 2-hour intervals continuously for a period of 48 hours.

When the weighings were completed, the leaves of all experimental plants were removed and measured. The length and width in millimeters were determined for each leaf. Width measurements were made at the point of greatest width. Blueprints were made of a large number of leaves of each of the varieties, and the areas were determined through the use of a planimeter (fig. 1). The areas thus obtained were compared with those obtained empirically by multiplying the product of two times (length × width) by the factors 0.75, 0.82, and 0.825. Although the factors giving the best fit for each variety varied slightly, it was apparent after many trials that the formula $2(L \times W \times 0.8)$ gave areas very closely approximating those obtained

by planimeter measurements. That formula therefore was used in calculating leaf areas throughout the experiments.

The areas of the stems were determined by multiplying the height (including the head) by the diameter at midheight times 3.1416. The figures thus obtained probably were not entirely accurate because of the inclusion of the heads, which obviously have a greater area than the same length of stems. However, it is known that most wheat stems taper toward the base and toward the head, having the greatest diameter at midheight. Taking diameter readings at that point, therefore, compensated partly for the greater area of the heads.

In calculating the area of transpiring surface, each jar was considered a unit. The sums of the areas of all leaves and stems of the five plants in each jar were calculated and reduced to terms of square meters. By using the weights of water lost during the experimental periods and the transpiring areas of the plants, therefore, it was possible to calculate the water lost per unit of area.

EXPERIMENTAL RESULTS

EFFECT OF LEAF RUST INFECTION ON TRANSPIRATION OF PLANTS OF DIFFERENT AGE

Preliminary experiments were conducted during the greenhouse season of 1933–34 to ascertain the methods most useful for later experiments. Two jars of each variety of wheat were inoculated with leaf rust at each of four stages of growth, viz, the seedling, jointing, booting, and flowering stages. Two similar jars were retained as controls at each stage of growth. Each jar contained five plants, and the four units of each experimental group were selected for similarity of water losses on the basis of their behavior previous to the time of hourly readings.

When leaf rust infection was fully developed on the inoculated plants, the four jars of each variety were brought to their equalized weight early in the morning and weighed hourly thereafter for various periods. The plants tested in the seedling stage were weighed each hour from 9 a. m. to 9 p. m. on January 20 and from 8 a. m. to 4 p. m. on January 21—an elapsed time of 31 hours. The plants studied in the other three stages of growth were weighed hourly from early morning until late evening of 1 day and given a final weighing at 9 a. m. the following morning. The elapsed times for the groups tested in the jointing, booting, and flowering stages were 23, 24, and 23.5 hours, respectively. The amount of water lost through transpiration was recorded at the time of each weighing. The data obtained are presented in summarized form in table 1.

On the basis of the water lost per square meter of transpiring surface, rust infection apparently caused a considerable increase in the transpiration of plants in the seedling, jointing, and heading stages of growth. However, there was only a slight increase in Pusa No. 4 and a slight decrease in Pusa 52 × Federation in the booting stage. The reason for this is not clear, but it probably was due to the small number of experimental units. This is further indicated by the lack of correlation between the amount of rust infection and the percentage increase in the quantity of water lost by infected plants. For example, in the seedling stage of growth, 14.86 percent rust in Pusa No. 4 apparently resulted in an increase of 28.19 percent in transpiration, while only 6 percent infection on Pusa 52 × Federation resulted in a 36.19 percent

increase. In the jointing stage of growth the lack of correlation between amount of rust and loss of water was still more marked. Only in the experiments during flowering did the percentage increase in transpiration seem reasonable for the amount of infection present on the susceptible variety and the absence of infection on the resistant host. There was a moderate development of uredia on the resistant variety in the early stages of growth. However, in later stages infection appeared as copious flecking accompanied by a few very small uredia.

TABLE 1.—*Effect of leaf rust infection on the transpiration of plants of Pusa No. 4 (susceptible) and Pusa 52 × Federation (resistant) wheat at 4 stages of growth in the greenhouse at Manhattan, Kans., 1934*

Stage of growth and variety	Plant group	Experimental period: Dates	Experimental period: Hours	Average rust infection	Total transpiring area	Total water lost	Water lost per square meter	Increase or decrease in transpiration associated with rust infection
				Percent	*Square meters*	*Grams*	*Grams*	*Perc…*
Seedling stage:								
Pusa No. 4	Rusted	Jan. 20–21	31	14.86	0.561	777	1,385.0	+2…
	Rust-free			.00	.709	766	1,080.4	-----
Pusa 52 × Federation	Rusted			6.00	.664	994	1,496.9	+3…
	Rust-free			.00	.958	1,053	1,099.1	-----
Jointing stage:								
Pusa No. 4	Rusted	Feb. 5–6	23	40.91	.696	722	1,037.3	+1…
	Rust-free			.00	.755	664	879.5	-----
Pusa 52 × Federation	Rusted			1.21	1.027	765	744.9	+1…
	Rust-free			.00	.955	605	633.5	-----
Booting stage:								
Pusa No. 4	Rusted	Feb. 13–14	24	32.84	.732	707	965.8	+…
	Rust-free			.00	.787	735	933.9	
Pusa 52 × Federation	Rusted			(1)	.914	920	1,006.6	−…
	Rust-free			.00	.912	936	1,026.3	-----
Flowering stage:								
Pusa No. 4	Rusted	Feb. 23–24	23.5	39.76	.642	616	959.5	+2…
	Rust-free			.00	.621	494	795.5	-----
Pusa 52 × Federation	Rusted			(1)	.683	524	767.2	-…
	Rust-free			.00	.726	554	763.0	-----

[1] Trace.

It was apparent early in the studies that hourly weighings were u… satisfactory because the losses were so small that the error in weighin… was likely to be large. It also soon was apparent that plants in t… seedling, jointing, and booting stages of growth were not well adapt… to an experiment of this kind. The principal reason for this was t… impossibility of obtaining rust infection on all leaves. After plan… were inoculated, it was necessary to allow about 14 days for the ru… to reach full development before readings could be made. Duri… that period the plants put out new leaves and tillers bearing no inf… tion. This is well illustrated by the data in the last column in table… The percentages were obtained by dividing the area of rusted leaves… the total area of leaves from each group of inoculated plants.

These data clearly show that a large proportion of the leaves in t… seedling and jointing stages of growth had no infection whatev… Furthermore, it was observed that many of the leaves near the bas… of the plants during the early stages of growth were only partly acti… Many such leaves were brown and shriveled at the tips, and t… remaining living portions frequently were chlorotic. Leaves in th… condition were extremely difficult to measure accurately.

ABLE 2.—*Comparative number of leaves and percentages of leaf area infected with leaf rust on plants of 2 varieties of wheat inoculated at 4 stages of growth in the greenhouse at Manhattan, Kans., 1934*

Stage of growth and variety	Leaves			Area represented by infected leaves
	Total	Noninfected	Infected	
	Number	*Number*	*Number*	*Percent*
Seedling stage:				
Pusa No. 4	179	48	131	67.05
Pusa 52 × Federation	249	92	157	63.61
Jointing stage:				
Pusa No. 4	205	39	166	84.68
Pusa 52 × Federation	215	86	129	63.86
Booting stage:				
Pusa No. 4	193	28	165	89.68
Pusa 52 × Federation	174	36	138	88.87
Flowering stage:				
Pusa No. 4	106	0	106	100.00
Pusa 52 × Federation	116	0	116	100.00

The results shown in tables 1 and 2, and other observations made during the course of the experiments, indicated that it was impracticable to make weighings oftener than once every 2 hours to determine periodic water losses, and that plants in the flowering stage of growth could be expected to give the most reliable results. It also was apparent that more plants were necessary in each experimental group to decrease the error due to small numbers.

DIURNAL RHYTHM OF TRANSPIRATION IN THE WHEAT PLANT AS AFFECTED BY LEAF RUST INFECTION

One of the most striking features of the preliminary experiments was the sharpness of definition and regularity of the diurnal rhythm of transpiration. Regardless of the age of the plant, the rate of transpiration rose rapidly after 8 a. m. and reached its peak between 2 and 3 p. m. From that point the rate of transpiration declined rather rapidly for a time, then dropped suddenly to a very low point between 5 and 6 p. m. Although the plants were not weighed at intervals throughout the night in the preliminary experiments, the hourly weighings between 5 and 9 p. m. and those made early the following morning showed that the loss of water during the night was very slight as compared with that during midday. For example, nonrusted seedling plants of Pusa No. 4 lost 117.1 gm. of water per square meter of surface between 2 and 3 p. m. and only 9.8 gm. per square meter between 5 and 6 p. m. In the flowering stage of growth, noninfected plants of the same variety transpired 132.0 gm. per square meter between 2 and 3 p. m. and only 27.4 gm. between 5 and 6 p. m. The other variety behaved in a similar manner. It was noted, however, that the time of the sharp drop in transpiration came somewhat later for plants in the flowering stage than for those in the seedling stage. This presumably was due to the difference in the length of day at the time the readings were made. The seedling readings were made on January 20 and the flowering-stage readings were made on February 23, when there was about 45 minutes more daylight at the end of the day.

Although the experiments of 1933–34 were only preliminary in nature, they indicated that leaf rust definitely increased the transpiration of infected plants. There also was strong evidence that

rust infection was accompanied by proportionately greater losses o
water at night than during the day, as compared with the rust-fr
plants. Both of these points are well illustrated in table 3, whe
the transpiration of rusted and rust-free plants of two varieties o
wheat in the juvenile and adult stages of growth are given for su
cessive 2-hour periods from 10 a. m. to 8 p. m. It seems significa
that, with but two exceptions, the highest loss of water for both ruste
and rust-free plants in both stages of growth occurred during the perio
from noon to 2 p. m. It also is clear that transpiration was low durin
the first period in the morning and during the last two periods i
the evening.

TABLE 3.—*Loss of water through transpiration by juvenile and adult plants of t varieties of spring wheat at intervals of 2 hours during the day and night in t greenhouse at Manhattan, Kans., 1933–34*

Variety and stage of growth	Plant group	Rust infection	Water per square meter lost through transpirat during successive 2-hour periods ending a					
			10 a. m.	12 m.	2 p. m.	4 p. m.	6 p. m.	8 p.
		Percent	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gra*
Pusa No. 4:								
Juvenile	Rusted	14.86	21.4	185.4	224.6	183.6	48.1	4
	Rust-free	.00	28.2	152.3	167.8	173.5	28.2	2
Adult	Rusted	39.76	42.1	188.5	224.3	201.0	57.7	6
	Rust-free	.00	30.6	183.6	194.8	188.0	48.3	1
Pusa 52 × Federation:								
Juvenile	Rusted	6.00	27.1	230.4	224.4	191.3	60.2	34
	Rust-free	.00	20.9	154.5	156.6	173.3	47.0	2
Adult	Rusted	(1)	8.8	177.2	206.4	174.3	17.5	36
	Rust-free	.00	16.5	170.8	209.3	158.3	31.7	5

[1] Trace.

The effect of leaf rust infection on the rate of transpiration is show
to best advantage by the susceptible variety Pusa No. 4. In th
variety the rusted plants lost more water than the rust-free contro
at all readings except that made at 10 a. m. on juvenile plant
Although the readings for both rusted and rust-free plants were lo
at 8 p. m. as compared with those made at 2 p. m., the percentag
increase in transpiration in rusted plants over the rust-free contro
was much greater for the night than for the day reading. For exam
ple, the transpiration of rusted juvenile plants of Pusa No. 4 at
p. m. was 33.85 percent higher than that for the control plants, whil
at 8 p. m. the rusted plants transpired 93.39 percent more than th
controls. In the adult plants of the same variety at flowering tim
the rusted plants transpired 15.14 percent more than the controls a
2 p. m. and 362.07 percent more than the controls at 8 p. m.

The difference between day and night increases in transpiration du
to rust infection was not so striking in Pusa 52 × Federation. Ruste
juvenile plants of that variety transpired 43.29 percent more than th
rust-free controls at 2 p. m. and 50.43 percent more than noninfecte
plants at 8 p. m. It should be kept in mind that the development o
uredia on infected plants of this group was only 6.0 percent. Th
transpiration of the rust-free adult plants was higher than that of th
inoculated plants at both the 2 p. m. and 8 p. m. readings. Rus
infection at that stage of growth was represented mostly by flecking
with only occasional small uredia.

CUMULATIVE AND PERIODIC TRANSPIRATION OF RUSTED AND RUST-FREE WHEAT PLANTS

The preliminary experiments indicated that leaf rust infection caused an increase in the rate of transpiration of wheat plants but suggested the need for greater numbers of plants and some refinements in technique. During the greenhouse season of 1934–35 the experiments were repeated, with 20 jars each of Pusa No. 4 and Pusa 52 × Federation. Each jar contained 5 plants. No inoculations were made until all plants had reached the flowering stage. At that time plants in 10 jars of each variety were heavily inoculated with leaf rust. The inoculated plants were placed in a moist chamber for 24 hours and then removed to the greenhouse bench. The control plants of each variety received the same treatment as the experimental ones except that they were not inoculated.

When leaf rust infection was fully developed, all dead and partly dead lower leaves, as well as all small weak tillers, were removed from the experimental plants. All jars were brought to original weight at 8 o'clock the following morning. For 48 hours the loss of water through transpiration was recorded by weighings made at intervals of 2 hours except that no readings were made from midnight until 8 a. m. Cumulative losses for the latter period were recorded. The data obtained are shown in table 4 and figures 2 and 3.

TABLE 4.—*Effect of leaf rust infection on the transpiration of plants of Pusa No. 4 and Pusa 52 × Federation wheat during a 48-hour period in the greenhouse at Manhattan, Kans., 1935*

Variety	Plant group	Average[1]				Increase in transpiration due to rust
		Rust infection	Transpiring area	Water lost	Water transpired per square meter	
		Percent	*Square meter*	*Grams*	*Grams*	*Percent*
Pusa No. 4	Rusted	67.01	0.3059	547.2	1,788.8	13.17
	Rust-free	.00	.3193	504.7	1,580.6	------
Pusa 52 × Federation	Rusted	2.54	.3282	553.6	1,686.7	5.05
	Rust-free	.00	.3428	550.4	1,605.6	------

[1] Per jar of 10 jars containing 5 plants each.

The average loss of water per square meter of transpiring surface in the susceptible Pusa No. 4 was increased 13.17 percent by heavy leaf rust infection, as shown in table 4. At this time the infected plants had an average rust reading of 67.01 percent. The infected plants of Pusa 52 × Federation transpired 5.05 percent more water than the rust-free controls, although the average rust reading of infected plants was only 2.54 percent. A comparison of the increase in transpiration in the two varieties with the rust readings indicates that the increase was proportionately greater in the resistant variety. It must be remembered, however, that the rust readings were based only on the number of uredia present, although there was abundant flecking in addition to the uredia on the leaves and leaf sheaths of the resistant variety. Earlier experiments (*4*) have shown that heavy flecking increases the water requirement of wheat plants, and undoubtedly it also causes an increase in the transpiration.

The total amount of water lost through transpiration during any experimental period is of less interest than the cumulative and periodic water losses, which show when they occur. Figure 2 shows the total cumulative water losses of rusted and rust-free plants of both varieties of wheat studied in 1934–35. During the first test day the total transpiration of the rusted and rust-free groups of Pusa No. 4 was about the same from 8 a. m. to 6 p. m. After the latter hour the rust-free controls transpired very slowly until early the following morning, while the rusted plants continued to lose considerable water,

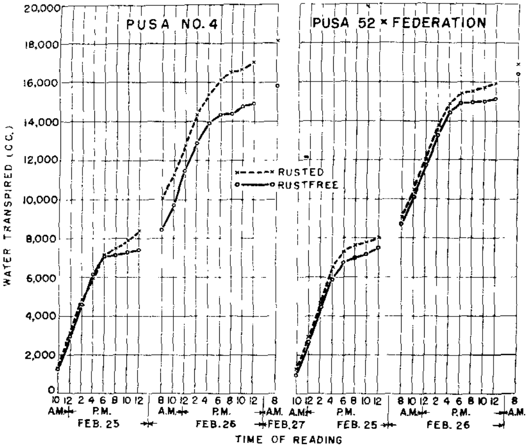

FIGURE 2.—Cumulative losses of water through transpiration by 50 rusted and 50 rust-free plants each of Pusa No. 4 (susceptible) and Pusa 52 × Federation (resistant) during 2 days in 1935.

although the rate was less than that shown during the day. During the second day the results were similar, the rusted plants continuing to transpire more rapidly than rust-free plants after 6 p. m. The trend is shown to be much the same with the resistant variety except that the differences between rusted and rust-free plants are considerably less.

The periodic losses recorded in 1934–35 are shown graphically in figure 3. These show the sharp rise in the rate of transpiration of all plants from early morning to midday and the equally sharp drop after about 4 p. m., regardless of variety or rust infection. The higher rate of transpiration of the rusted as compared with the nonrusted plants of Pusa No. 4 during the night is particularly clear in the readings of February 25. On the following night, except for the 10 p. m. reading, they also transpired more than the rust-free controls for all periods from 6 p. m. to midnight. The small difference

n the transpiration of the rusted and rust-free groups of Pusa 52 × Federation is shown clearly in figure 3 as well as in figure 2.

The experiments of 1934–35 showed rather conclusively that leaf ust infection caused considerable increase in the rate of transpiration of wheat plants of a susceptible variety and to a lesser degree of a esistant variety. They also indicated that one of the principal easons for the increase was a higher rate of transpiration in rusted plants during the night.

It was desired to repeat the experiments, however, after making a ew refinements in procedure. During the greenhouse season of 935–36, further studies of the problem were made, in which the

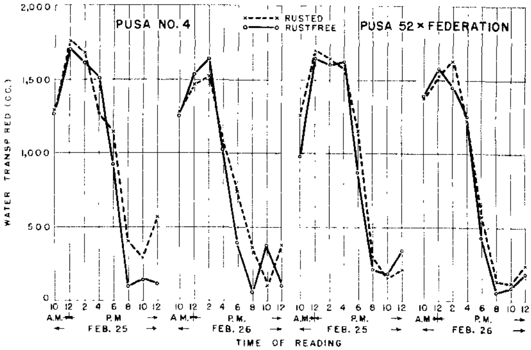

FIGURE 3.—Periodic losses of water through transpiration by 50 rusted and 50 rust-free plants each of Pusa No. 4 (susceptible) and Pusa 52 × Federation (resistant) at 2-hour intervals during 2 days in 1935.

awnless variety Reward was substituted for Pusa 52 × Federation with the thought of eliminating any differences that might have been due to the awns of the latter. The plants were grown in the same containers and handled in the same manner as in the 2 preceding years. At flowering time, 6 jars with Pusa No. 4 and 6 with Reward were selected for the final experiment. These jars were nearly identical in their rate of water loss through transpiration up to the beginning of the experimental period.

When the plants had reached the desired stage of growth, 3 jars (15 plants) of each variety were placed in the moist chamber and heavily inoculated with leaf rust. The 3 control jars of each variety also were placed in the moist chamber but were not inoculated. After rust infection was fully developed, all senescent lower leaves and all but the 3 main tillers on each plant were removed. There remained 5 plants in each jar, each plant having 3 vigorous tillers and 12 active green leaves. The plants were allowed to stand 24 hours after trimming, in order to prevent the recording of any losses due to evaporation from freshly cut surfaces, after which all jars were brought

to their equalized original weights. They were then weighed at the end of each 2-hour period for a continuous 48-hour period. The results are shown in table 5 and figures 4 and 5.

TABLE 5.—*Effect of leaf rust infection on the transpiration of plants of Pusa No. 4 and Reward spring wheat during a 48-hour period in the greenhouse at Manhattan, Kans., 1936*

Variety	Plant group	Average[1]				Increase or decrease in transpiration due to rust
		Rust infection	Transpiring area	Water lost	Water transpired per square meter	
		Percent	*Square meter*	*Grams*	*Grams*	*Percent*
Pusa No. 4	Rusted	57	0.8779	444.3	506.0	+32.7
	Rust-free	0	.9044	345.0	381.4	
Reward	Rusted	1	.8629	312.6	362.2	−1.[illegible]
	Rust-free	0	.8555	315.0	368.2	

[1] Per jar of 3 jars containing 5 plants each.

The data presented in table 5 show that heavy leaf rust infection on Pusa No. 4 resulted in an increase of 32.7 percent in transpiration during the 48-hour period. This is nearly three times greater than the increase observed in 1935, although the percentage of rust infection was higher in 1935 than in 1936. However, in 1936 every leaf on the inoculated susceptible plants bore abundant uredia of leaf rust, while in 1935 there were some leaves on all plants that had little or no infection. Also, the difference may be partly due to the smaller number of experimental units used in 1936. This possibility is suggested by the very small decrease in the transpiration of infected plants of the resistant variety (Reward). The important point, however, is that in both 1935 and 1936 heavy leaf rust infection resulted in a considerable increase in the rate of transpiration of the susceptible plants, while that of the resistant plants was affected but little by the rust. These points are graphically illustrated in figures 4 and 5.

Figure 4 shows the total cumulative weight of water lost per square meter of transpiring surface by both varieties during the 48-hour experimental period in 1936. It shows to excellent advantage the gradual increase and the ultimate wide difference in the amount of water transpired between rusted and rust-free plants of Pusa No. 4 as well as the slight difference between similar groups of Reward. It also verifies the observation made in 1935 that the principal increase in the rate of transpiration of rusted over nonrusted plants of a susceptible variety occurs during the night.

The diurnal nature of transpiration in wheat and its modification by leaf rust infection is shown in figure 5. The readings made in 1936 are complete because weighings were made regularly every 2 hours for the entire 48-hour period. Figure 5 shows the transpiration of rusted and rust-free plants of both varieties during each 2-hour period of the experiment. The transpiration of both rusted and rust-free plants rose very rapidly from 8 a. m. until noon, remained high until about 4 p. m., then declined rapidly. The rusted plants of the susceptible variety, except for one reading on January 24, transpired

more at all times than rust-free plants. On the other hand, the control plants of Reward sometimes surpassed the infected plants in

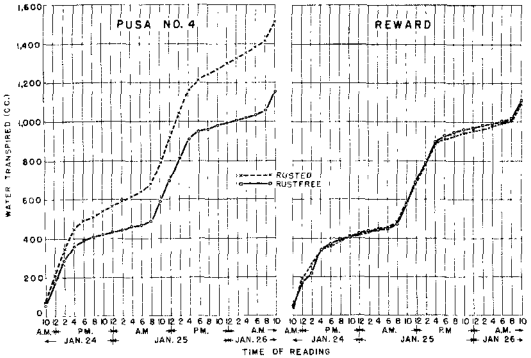

FIGURE 4.—Cumulative losses of water through transpiration by 15 rusted and 15 rust-free adult plants each of Pusa No. 4 (susceptible) and Reward (resistant) spring wheat during a 48-hour period in 1936.

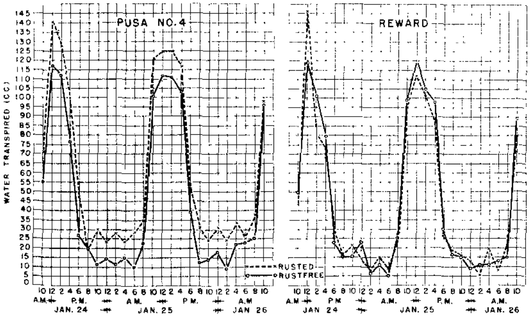

FIGURE 5.—Periodic losses of water through transpiration by 15 rusted and 15 rust-free adult plants each of Pusa No. 4 (susceptible) and Reward (resistant) spring wheat at 2-hour intervals for a 48-hour period in 1936.

transpiration. The fact that the rusted plants of the susceptible Pusa No. 4 transpired more at night than the rust-free controls is shown in both figures 4 and 5.

COMPARATIVE TRANSPIRATION OF RUSTED AND RUST-FREE WHEAT PLANTS DURING PERIODS OF LIGHT AND DARKNESS

Although figures 2, 3, 4, and 5 indicate that the transpiration of rusted wheat plants is higher during the night than that of nonrusted plants, the data in tables 6 and 7 throw further light on the matter. In 1935 and 1936, jars containing rusted and rust-free plants were weighed at intervals for 48 hours. The readings were made during February in 1935 and during January in 1936. At that time of the year darkness begins at approximately 6 p. m. and ends at about 7 a. m. Observations had shown that normal transpiration in the wheat plant began a rapid rise at about 8 a. m. each day and was low and nearly stationary after 6 p. m. It seemed logical, therefore, to consider the period from 8 a. m. to 6 p. m. a period of light, and that from 6 p. m. to 8 a. m. a period of darkness. Thus, there was a 10-hour period of high transpiration and a 14-hour period of low transpiration each day.

It seems desirable for several reasons to present the data obtained for each period of light and darkness rather than to total or average the figures for the 2 days. In the first place, it should be emphasized that the plants were grown in relatively small containers and that no water was added during the 2-day period. The amount of water available for plant use therefore was steadily declining. That fact alone might conceivably affect the results. The data in table 6 show that less water was transpired by both rusted and rust-free plants of both varieties on the second day than on the first day. However, that apparently had no effect on the difference in the rate of transpiration of rusted and rust-free plants.

TABLE 6.—*Average transpiration per jar of 10 rusted and 10 rust-free jars each of 2 varieties of spring wheat during 2 days and 2 nights in the greenhouse at Manhattan, Kans., 1935*

Variety	Plant group	Rust infection	Average transpiring area	Water lost per square meter of transpiring surface			
				8 a. m. to 6 p. m.		6 p. m. to 8 a. m.	
				First day	Second day	First day	Second day
		Percent	*Square meter*	*Grams*	*Grams*	*Grams*	*Grams*
Pusa No. 4	Rusted	67	0.3059	716.34	609.48	260.46	201.96
	Rust-free	0	.3193	704.70	588.12	142.01	147.34
Pusa 52 × Federation	Rusted	3	.3283	732.62	633.84	176.52	144.82
	Rust-free	0	.3428	673.18	609.06	183.99	138.48

Another factor that is known to affect the transpiration of wheat plants in the greenhouse is the variability in greenhouse conditions from day to day. Plants transpire more on bright days, when temperatures are high and the greenhouse ventilators must be open, than on cool, cloudy days when the ventilators are closed. Table 7 shows greater transpiration for the second day in 1936, which was warm and bright, than for the first day, which was cooler and cloudy. This, however, had no influence on the relative rates of transpiration of rusted and rust-free plants. The difference in the transpiration levels in the two seasons was due partly to environmental differences and partly to differences in growth and vigor of plants.

TABLE 7.—*Total transpiration of groups of 15 rusted and 15 rust-free plants each of Pusa No. 4 and Reward spring wheat during day and night periods of 2 consecutive days in the greenhouse at Manhattan, Kans., 1936*

Variety	Plant group	Rust infection	Transpiring area	Water lost per square meter of transpiring surface			
				8 a. m. to 6 p. m.		6 p. m. to 8 a. m.	
				First day	Second day	First day	Second day
		Percent	*Square meter*	*Grams*	*Grams*	*Grams*	*Grams*
Pusa No. 4	Rusted	57	0.8779	486.4	541.0	186.8	206.2
	Rust-free	0	.9044	388.1	464.4	99.5	107.2
Reward	Rusted	1	.8629	372.0	426.5	107.8	100.8
	Rust-free	0	.8555	373.0	453.5	99.3	90.0

Table 6 shows that rusted and rust-free plants of Pusa No. 4 transpired nearly the same during the two daylight periods, but that rusted plants lost much more water than the rust-free controls during the periods of darkness. In the resistant Pusa 52 × Federation, however, there was little difference between rusted and rust-free plants during either of those periods.

The data obtained in 1936, as shown in table 7, corroborate those obtained in 1935. In both years the rusted plants of the susceptible variety transpired nearly twice as much at night as nonrusted plants, while there was little difference in the nighttime transpiration of infected and noninfected plants of the resistant variety.

While the figures presented in tables 6 and 7 show that rusted plants of a susceptible variety transpire much more during the night than do rust-free plants of the same variety, they do not show the difference for comparable time periods. The daylight periods were only 10 hours, while the periods of darkness were 14 hours. The data presented in table 8 therefore were calculated on the basis of the transpiration per square meter per hour. This obviously causes wider differences in transpiration between the periods of daylight and darkness than those shown in tables 6 and 7.

Although only data for 1935 are shown in table 8, the results for 1936 were similar. It will be noted that the difference between the transpiration of rusted and rust-free plants of both varieties was very small during the daylight periods, although rust apparently caused a slight increase in all cases. In the resistant variety the transpiration during the night was much lower than that during the day, but there was not much difference between the rate of transpiration of rusted and rust-free plants as shown by the percentages of increase or decrease.

The most outstanding feature of the data shown in table 8 is the enormous percentage increase in the nocturnal transpiration of rusted plants of Pusa No. 4 over the rust-free controls of that variety. During the first night the rusted group transpired 83.41 percent more than the nonrusted group, but during the second night the increase was only 37.08 percent. The lower percentage probably was due to the smaller amount of water available for transpiration as the plants neared the end of the 48-hour period. For the two periods of darkness the rusted plants of Pusa No. 4 therefore transpired an average of

60.24 percent more per square meter per hour than the rust-free controls. The difference between the rates of transpiration of heavily rusted and rust-free plants at night was still more marked in 1936, when the rusted plants of Pusa No. 4 lost on an average 89.62 percent more water per square meter per hour than did rust-free plants.

TABLE 8.—*Transpiration per square meter per hour in rusted and rust-free plants of a susceptible and a resistant variety of wheat during 2 periods each of daylight and darkness in the greenhouse at Manhattan, Kans., 1935*

Variety and plant group	Transpiration per square meter per hour			
	8 a. m. to 6 p. m.		6 p. m. to 8 a. m.	
	First day	Second day	First day	Second day
Pusa No. 4 (susceptible).	*Grams*	*Grams*	*Grams*	*Grams*
Rusted	716.34	609.48	186.04	144.27
Rust-free	704.70	588.12	101.43	105.24
	Percent	*Percent*	*Percent*	*Percent*
Increase	+1.65	+3.63	+83.41	+37.08
Pusa 52 × Federation (resistant).	*Grams*	*Grams*	*Grams*	*Grams*
Rusted	732.62	633.84	126.09	103.44
Rust-free	673.18	609.06	131.42	98.91
	Percent	*Percent*	*Percent*	*Percent*
Increase or decrease	+8.83	+4.07	−4.05	+4.58

It seems clear from the foregoing data and discussion that the increase in the transpiration of rusted wheat plants of a susceptible variety over that of rust-free plants is very largely due to the higher rate of transpiration of the rusted plants during the night. The reasons for the higher nighttime rate of transpiration of rusted plants are speculative. Graf-Marin (*3*) and Gassner and Goeze (*2*) both suggest that higher nocturnal transpiration was partly due to transpiration of the rust fungus. Graf-Marin worked with a mildew that does not rupture the host-plant tissues as do the rust fungi. He also observed that the disease stimulated stomatal action in the host plant in a way that would favor higher transpiration. The studies reported herein throw little light on the causes of higher nocturnal transpiration in heavily rusted wheat plants. However, the large amount of ruptured plant tissue in such plants strongly suggests that transpiration through the ruptures caused by the uredia may be an important factor. The large amount of fungus tissue exposed in rust uredia also suggests that the fungus itself may be the cause of considerable loss of water. This loss of water by evaporation from thin-walled fungus cells can hardly be considered a function of the host plant but obviously is inseparable from normal transpiration. Two years' observations, therefore, suggested that the higher transpiration of rusted plants during the night was due partly to injury caused by the rupturing of the epidermis of the host plant by rust uredia and partly to transpiration by the fungus itself. That the latter is an important factor is suggested by the fact that the average transpiration of infected resistant plants during the night was only slightly higher than that of the noninfected controls.

The difference between the transpiration of rusted and rust-free plants of both resistant and susceptible varieties during daylight hours

was small. This probably was due to the very high transpiration level during the day. In all instances, however, the average transpiration of infected plants was slightly higher than that of the control plants during the day.

SUMMARY

The effect of leaf rust infection on the transpiration of a resistant and a susceptible variety of spring wheat was studied in the greenhouse during 3 years.

Plants were grown to maturity in sealed stone jars and leaf and stem areas were calculated. Blueprints made of representative wheat leaves proved that the formula $2(L \times W \times 0.8)$ gave leaf areas very near planimeter readings.

Plants in the flowering stage were found to be superior to younger plants or seedlings for studies of this nature.

Normal transpiration in the wheat plant was found to rise very rapidly during the morning hours, reach a maximum about noon, and decline rapidly in the late afternoon, reaching a very low point about 6 p. m. and remaining extremely low during the hours of darkness.

Observations were made on the transpiration of rusted and rust-free plants at 2-hour intervals for 48-hour periods in 1935 and 1936. It was found that rusted plants of the susceptible variety transpired 13.17 percent and 32.7 percent, respectively, more than nonrusted controls for the entire period in the 2 respective years.

The diurnal rhythm of transpiration was seriously disturbed in rusted plants of the susceptible variety. Such plants transpired much more than rust-free controls during the night. In 1935 the increase in nocturnal transpiration of rusted plants over the controls was 83.41 percent, while in 1936 it was 89.62.

The higher rate of transpiration of rusted plants at night apparently was due partly to transpiration through ruptures in the cuticle caused by the uredia and partly to the transpiration of the fungus itself.

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THE INFLUENCE OF THE AWNS UPON THE RATE OF TRANSPIRATION FROM THE HEADS OF WHEAT[1]

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INTRODUCTION

Although the exact physiological role of awns is not well established, it is generally considered that under certain climatic conditions the awns of wheat are useful structures. On this account awned varieties of wheat have been selected and grown for the most part in regions of limited rainfall. The data which have been obtained relative to the functions of the awns are frequently fragmentary, contradictory, and sometimes the result of questionable methods. Work has been in progress since 1935 at the Kansas Agricultural Experiment Station to try to determine some of the functions of the awns of wheat. The data herein reported, relative to the influence of the awns on the rate of transpiration from the heads, are thus only a portion of those obtained in the general study of the function of the awns of the wheat plant.

REVIEW OF LITERATURE

The quantity and quality of the grain produced has been the measure, for the most part, of the effects of the removal of the awns from cereals. Only a relatively few of the investigators have studied the influence of the awns on the rate of transpiration. Zoebl and Mikosch (*10*) [4] stated that the awns of barley are definite organs for transpiration. The awned heads transpired four to five times more water than the awnless heads. At the time of the functioning of the awns about one-half of the total transpiration of the plant was from the heads. The transpiration of the awns was most intense at the time of the greatest migration of reserve materials into the grain. An unsigned article in a German publication (*1*) stated that the transpiration from barley heads is decreased by the removal of the awns.

Vasilyev (*9*) in Russia studied the function of awns in rye, wheat, and barley. In experiments with Beloturka wheat, he found that the awns transpired 63.3 percent of the water evaporated by the heads, and in another variety 60.3 percent of the total transpiration of the heads was through the awns. The maximum transpiration of the awns was at flowering.

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[2] A portion of the thesis submitted by the senior author to the Graduate Council, Kansas State College of Agriculture and Applied Science, in 1937 for partial fulfillment of the requirements for the degree of master of science.

[3] The authors are especially indebted to C. O. Johnston, associate pathologist, U. S. Department of Agriculture, for selecting and supplying the wheat used in this study and for his helpful suggestions and criticisms.

[4] Italic numbers in parentheses refer to Literature Cited, p. 457.

Schmid (7) stated that the awns are actively concerned in transpiration, assimilation, and respiration. The de-awning of a wheat plant lowered its transpiration 10 to 30 percent. He found that the awned heads transpired relatively more water by night than by day, while with awnless heads as well as with the lamina of the leaf, the reverse was true. Since the awns and the lamina both have numerous stomata, Schmid considered that the stomata of the lamina opened wider during the day than did those of the awn.

Perlitius (6) found that the awned heads of wheat and barley showed a higher transpiration rate than the awnless heads. The same fact was reported by Conti (2) for the heads of durum wheat.

Schulze (8) showed that awned barley plants transpired much more than those which had been de-awned or were naturally awnless. The peak of transpiration was reached when the grain was in the milk stage.

METHODS OF EXPERIMENTATION

CULTURAL METHODS

The wheat plants were grown in glazed stone jars of 1-gallon capacity with the same amount of soil mixture firmly tamped into each jar. The soil mixture was made by adding 1 part sand to 4 parts of fertile loam soil. Sufficient water was added to this mixture to bring the moisture content to 45 percent of the water-holding capacity of the soil. The method used to facilitate the distribution of water throughout the soil in the jars and to prevent its loss from the surface was the same as that described by Johnston and Miller (4).

Before planting, a ½-inch cork borer was heated and used to make five equidistant holes in the paraffin layer above the soil. A germinating grain of wheat, with a radicle one-fourth of an inch long, was planted in the soil beneath each of these holes. The seeds were planted on October 25, 1935, and on November 2, 1936. At regular intervals, varying according to the stage of growth, the jars were weighed and sufficient water was added in each case to bring them to their original weight.

During the experimental work in 1935–36, it was found that a hybrid wheat of Indian origin, Pusa 52 × Federation, because of its type and rapidity of growth, and other characteristics, was the most desirable plant to use. It was used, therefore, exclusively in the experiments of 1937. The general appearance of these plants when they had attained their full vegetative growth is shown in figure 1.

SELECTION AND DE-AWNING OF HEADS

In the experiments of 1935–36 no record was kept of the development of the heads so their exact stage of growth at any given time was not known. Thus during 1935–36, the pairs of heads used in the transpiration experiments were matched as closely as possible only with regard to size. During 1936–37, however, a detailed record of 215 heads was kept so that in all the experimental work for that year the heads would be not only of the same size, but also of a known age and stage of development.

When the awns protruded visibly from the boot, the culm of the head was tagged and detailed notes were taken thereafter at 2-day intervals. Some heads were used for observational purposes only,

and spikelets were removed from these from time to time to determine the progress or rate of grain formation. Thus it was not necessary to remove any spikelets from the heads used in the transpiration studies to determine their stage of development.

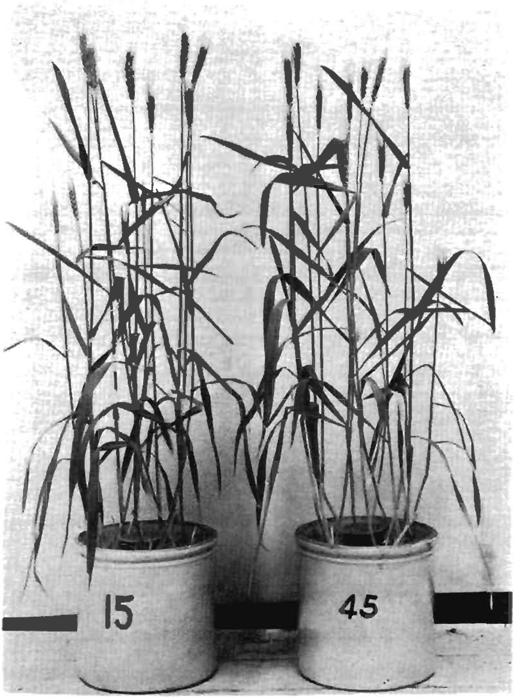

FIGURE 1.—Typical jars of Pusa 52 × Federation wheat, showing the condition of the plants on February 8, 1936, when the plants were 75 cm. in height. The planting date was October 25, 1935.

Four apparently similar heads were selected for each experiment. Two heads were used as controls, while the awns were clipped from the other two with small, sharp scissors. The appearance of the awned and de-awned heads at the time of an experiment can be observed in figure 2.

FIGURE 2.—Totally de-awned and intact awned beads of Pusa 52 × Federation wheat.

The objection may be raised that the injury resulting from the removal of the awns might influence transpiration to some extent, and that the lowering of the transpiration of the de-awned heads might be due to this effect and not to the loss of the awns. There is no means of measuring this effect, but after a period of 2 months when the wounds incident to removal had been healed for a long time, the transpiration from the de-awned heads was still as low as, or lower than it was at the beginning of the experiment.

METHOD OF MEASURING TRANSPIRATION

In these experiments, a modification of the Freeman (*3*) method was used in determining the rate of transpiration of the heads. The general procedure is as follows: An aspirator provides the suction that draws the air over the plant parts which are enclosed in a glass chamber and through weighed U-tubes containing a water absorbent. In the present experiments granular calcium chloride was used as the absorbent. The air that passes over the plant parts contains both the moisture released from these parts and that which is normally present in the air. The amount of moisture in the air at the time of the experiment is determined by the use of a control apparatus, figure 3, *h*, *m*, in which the same amount of air as is drawn over the plant parts is drawn through the U-tubes of the control apparatus, and the moisture contained therein is absorbed. The weight of the moisture collected in the control apparatus is then deducted from the amount found in the air which passed over the plant parts. The remainder is the weight of the water given off from the portion of the plant used in the experiment.

The apparatus used in 1936–37 is shown in figure 3. Three units were operated simultaneously in each experiment: One, *m*, a control, for determining the water content of the air, another, *n*, for determining the loss of water from the awned intact heads, and a third, *o*, for determining the water loss from the de-awned heads. In figure 3 the unit for the control, *h*, *e*, *m*, is shown at the extreme left. At the center, *i*, *e*, *n*, and right, *j*, *e*, *o*, are the units which determine the rate of transpiration of two awned heads and two de-awned heads, respectively.

The glass chamber (fig. 4) used was a type of small specimen jar. This chamber was 7 by 2½ inches, outside measurements, and had a capacity of 500 cc. It was fitted with a No. 8 three-holed rubber stopper split into two portions so that the line of cleavage passed through two of the holes to permit the introduction of the peduncles of the heads. In the third hole the outlet tube was inserted. The holes that held the two peduncles were sufficiently large to allow free intake of air around these parts when suction was applied by the aspirator to the outlet tube. The portions of the peduncles that protruded into the glass chamber were coated with vaseline to prevent transpiration, and when they were taken from the chamber this coating was removed to prevent injury to the peduncles.

A double set of the U-tubes was placed in connection for each unit (fig. 3, *e*, center), but only one pair was open during any one experiment. At the end of one experiment, the used pair of U-tubes was closed and a new experiment was started immediately by opening the unused pair of tubes. During the second experiment, the used pair

was removed for weighing and a new set was installed in the system for a later experiment.

Without the use of such a set-up, it would have been necessary to remove the three pairs of U-tubes and to install new ones at the end of each experiment. Under such conditions, the heads must be removed from the glass chambers or other special precautions must be taken to prevent an accumulation or condensation of moisture within these chambers. By the use of the extra pair of U-tubes, as above described, it was possible to make as many as four 1-hour determinations of

FIGURE 3.—The modified Freeman apparatus used in the study of the transpiration of wheat awns: *a*, Large aspirators; *b*, small aspirators; *c*, hose for filling aspirators; *d*, trough for carrying water from aspirators; *e*, U-tubes; *f*, atmometer; *g*, pinch-cocks of aspirators; *h*, glass chamber for control; *i*, glass chamber for awned heads; *j*, glass chamber for de-awned heads; *k*, plants bearing awned heads; *l*, plants bearing de-awned heads; *m*, control unit; *n*, *o*, units used for water determinations on head of wheat.

transpiration without disturbing the heads or pausing between experiments.

Two 18-liter aspirators, *a*, and one 4-liter aspirator, *b*, were used in each of the units. As the water siphoned from the aspirators, it emptied into a trough, *d*, and was carried by it to a nearby drain. The aspirators were refilled by a garden hose attached to the water line, *c*. The small aspirators were also connected into the system leading from the glass chambers and these were run while the heads were being inserted and the control was being set up. This procedure prevented an accumulation or condensation of moisture within the chambers during that time.

When arrangements were completed for beginning the experiment, the three small aspirators were closed, each pair of U-tubes, *e*, in each unit was opened, and one of the 18-liter aspirators, *a*, for each unit

as started. The time of starting the experiment was then recorded nd the porous-cup atmometer, *f*, was read. When the first aspira-ors were empty, the rubber tubes connecting them with the system ere clamped with pinchcocks and the second aspirator of each nit was started immediately. While the second aspirators were inning, the first ones were refilled, primed, and reconnected to the stem so that they could be started again as soon as they were eeded. By this arrangement a constant suction could be provided iroughout the experiment. In most of the experiments each of the vo aspirators of a unit was emptied twice, so that 72 liters of water ere used. As the 72 liters of water siphoned from the aspirator, 2 liters of air were necessarily rawn in to replace them. In rder to reach the aspirator the air ad to enter around the peduncles t the base of the heads (fig. 4), ow upward around the heads, own the outlet tube, and through ie weighed U-tubes (fig. 3, *e*) before could enter the aspirator where ie vacuum was created.

Since it took approximately 15 iinutes for one aspirator to empty, ie experiment had a duration of out 1 hour. By adjusting the rew clamps, *g*, *h*, on the outlet ibe of the aspirators, the units uld be made to finish at approxi-ately the same time. If the periment lasted slightly more or ss than 1 hour, the transpiration dues were reduced to that of an urly period. At the close of each periment, the atmometer, *f*, was ad and its evaporation likewise lculated to the hourly basis.

At the beginning of a series of periments, the amount of trans-ration from two pairs of intact ads was determined for a given riod of time. The ratio of the te of transpiration of one pair of ads to that of the other was then lculated. In this paper, this ratio is termed the "initial ratio" transpiration. After this initial ratio had been determined, one ir of heads was de-awned (fig. 2) and its rate compared with that the intact pair in numerous determinations.

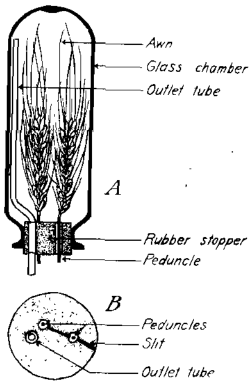

FIGURE 4.—*A*, Diagrammatic drawing of the glass chamber used; *B*, surface view of the rubber stopper which was used in the glass chamber, showing the slit between two holes to permit insertion of the peduncles, and cross sections of the outlet tube and of the peduncles around which the air entered.

EXPERIMENTAL RESULTS

During 1935–36, fifty-five 1-hour determinations were made to st the effects of the removal of the awns on the transpiration of the ad. These experiments were of a preliminary nature, and through iem the technique used during 1936–37 was perfected. Although

the results of the experiments performed during the first year agree in general with those herein reported, they are not tabulated becau they were obtained under various methods of technique.

During the year 1936–37, one hundred and eight 1-hour dete minations of transpiration exclusive of those used in determining th initial ratio were made on the same variety of wheat as used the firs year. Experiments were conducted with seven sets of heads (table 1 The number of determinations on each set ranged from a minimum o 12 to a maximum of 21. The experiments on the sets ranged fror 15 to 35 days. Three of the sets began at or before the flowerin stage, two at the early milk stage, one at the milk stage, and th seventh at the soft dough stage, and all were continued until th grain was ripe. The detailed data of these seven sets are shown i table 1. The de-awned heads in all except one of the determination showed a decidedly lowered transpiration in comparison with th intact heads. The one determination that showed an increase i transpiration of the de-awned heads over that of the intact heads oc curred in set 6 from 10:30 to 11:29 a. m. on February 20, 1937. I that experiment the ratio of the transpiration of the de-awned head to that of the awned ones was 1.04. Since in 108 experiments thi is the only case in which the transpiration from the de-awned head exceeded that from the intact ones, it seems probable that some erro occurred.

Table 1 shows in detail the data that were obtained in each set c experiments. In this table are found the date of each experiment, th time of day that it was performed, the general stage of development c the head, the nature of the weather, the rate of evaporation from porous cup atmometer, the temperature, the number of days that ha elapsed from the time of de-awning until the experiment, the numbe of days since flowering, the hourly rate of transpiration from th de-awned heads and the awned heads and the ratio of these two rate of transpiration. Any detailed information relative to these experi ments can be obtained by a study of table 1.

Johnston and Miller (5) studied the influence of leaf rust (*Puccini triticina*) upon the rate of transpiration of wheat plants of the sam variety as that reported herein and at the same time that these experi ments were being conducted. One observation which was made b them at that time and which was not reported in their experiments wa that the amount of water transpired from the heads of wheat wa relatively small in comparison with the amount lost in the same tim from the entire plant. The heads transpired only from 1 to 5 percen of the water lost from the entire plant.

In figure 5, the determinations of transpiration are plotted accordin to the number of days after flowering. By this method of plottin the data, the amount of transpiration is correlated with the stage c development of a plant or plant part. For the most part, the de terminations have been grouped into 3-day units. The data wer obtained by averaging the results of all experiments occurring withi the time limit of any unit. Thus, in the unit 1 to 3 days after flowe ing, the values representing the transpiration of awned and de-awne heads are the averages of thirteen 1-hour determinations as take from the various sets of experiments (table 1).

Set No. and date	Temperature	Time of day [1]	Evaporation from atmometer per hour	Water in each 72 liters of air	Net water loss per hour: 2 awned heads	Net water loss per hour: 2 de-awned heads	Stage of development	Days from time of first flowering	Time after de-awning	Transpiration ratio of de-awned to awned heads	Weather
	° F.		*Cubic centimeters*	*Milligrams*	*Milligrams*	*Milligrams*		*Number*			
SET NO. 1											
Jan. 19	71	10:40–11:40	1.4	305.5	220.7	222.3	Flowering	0		[2] 1.01	Sunny.
Do	72	3:05– 4:12	1.4	391.3	161.3	113.0	do	0	5 minutes	.71	Cloudy.
Jan. 20	75	9:01–10:10	1.4	459.3	201.6	132.0	do	1	1 day	.65	Do.
Do	74	10:32–11:34	1.3	276.2	825.2	461.4	do	1	do	.56	Sunny.
Do	68	1:42– 2:44	1.1	318.5	376.2	280.5	do	1	do	.75	Cloudy.
Do	68	3:05– 4:05	1.0	448.7	184.4	132.5	do	1	do	.72	Do.
Jan. 21	73	9:20–10:24	1.4	471.3	183.1	155.4	do	2	2 days	.85	Do.
Jan. 22	73	8:17– 9:17	1.5	410.5	195.8	125.3	do	3	3 days	.64	Do.
Jan. 23	68	8:17– 9:21	1.3	428.1	167.1	120.8	do	4	4 days	.72	Sunny.
Jan. 24	78	8:02– 9:01	1.3	532.3	188.8	144.5	do	5	5 days	.77	Do.
Jan. 25	67	8:59– 9:57	.8	507.9	318.6	205.2	do	6	6 days	.64	Do.
Jan. 26	70	8:39– 9:40	1.3	447.0	200.2	172.0	do	7	7 days	.59	Do.
Jan. 27	67	8:04– 9:03	1.0	530.5	178.9	112.0	Between flowering and early milk.	8	8 days	.63	Do.
Do	70	9:03–10:04	1.4	433.5	338.8	188.7	do	8	do	.56	Do.
Do	72	2:59– 3:59	1.9	314.7	359.7	200.8	do	8	do	.56	Do.
Do	72	3:59– 5:00	1.0	446.5	213.5	125.6	do	8	do	.59	Do.
Jan. 28	63	7:55– 8:55	1.0	459.2	102.2	31.6	Early milk	9	9 days	.31	Cloudy.
Feb. 4	65	8:45– 9:44	1.0	406.2	294.8	123.6	Soft dough	16	16 days	.42	Sunny.
Feb. 8	74	12:02– 1:00	2.0	396.6	337.3	170.2	do	20	20 days	.50	Do.
Feb. 15	72	9:59–10:57	1.2	555.1	215.1	132.4	Hard dough	27	27 days	.62	Do.
Feb. 19	67	12:39– 1:37	.8	654.6	94.3	34.6	do	31	31 days	.37	Cloudy.
Feb. 22	75	8:45– 9:44	1.5	495.2	15.4	11.9	Grain ripe	34	34 days	.77	Do.
SET NO. 2											
Jan. 21	74	10:52–11:54	1.4	459.9	247.5	252.7	Flowering	0		[2] 1.02	Do.
Do	75	2:41– 3:41	1.2	494.7	202.2	130.9	do	0	6 minutes	.65	Do.
Do	70	3:41– 4:43	1.1	451.5	178.2	114.6	do	0	1 hour	.64	Do.
Jan. 22	72	9:34–10:35	1.5	393.5	221.6	188.9	do	1	1 day	.85	Sunny.
Jan. 23	68	9:40–10:40	1.4	404.2	314.6	235.9	do	2	2 days	.75	Do.
Jan. 25	73	10:11–11:11	1.5	475.2	349.0	226.7	do	4	4 days	.65	Do.
Jan. 26	73	1:26– 2:26	2.0	322.2	287.5	167.8	do	5	5 days	.58	Do.
Do	73	2:26– 3:27	1.7	309.7	331.2	196.2	do	5	do	.59	Do.
Feb. 4	71	9:57–10:55	2.2	363.7	366.9	238.3	Milk	14	14 days	.65	Do.
Feb. 8	70	1:00– 2:06	1.8	364.3	307.8	187.1	Soft dough	18	18 days	.61	Do.
Feb. 15	73	11:04–12:02	1.8	520.2	279.2	220.4	Hard dough	25	25 days	.82	Cloudy.
Feb. 19	70	1:43– 2:42	.9	646.4	109.1	81.2	do	29	29 days	.74	Do.
Feb. 22	77	9:49–10:49	1.9	391.9	262.0	122.6	Ripe	32	32 days	.47	Sunny.

See footnotes at end of table.

TABLE 1.—*Transpiration experiments, with 7 sets of awned and de-awned heads of Pusa 52×Federation wheat, in the greenhouse, Manhattan, Kans., 1937*—Continued

Set No. and date	Temperature	Time of day	Evaporation from atmometer per hour	Water in each 72 liters of air	Net water loss per hour: 2 awned heads	Net water loss per hour: 2 de-awned heads	Stage of development	Days from time of first flowering	Time after de-awning	Transpiration ratio of de-awned to awned heads	Weather
	° F.		Cubic centimeters	Milligrams	Milligrams	Milligrams		Number			
SET NO. 3											
Jan. 22	75	1:30– 2:30	1.5	404.6	374.1	319.9	Flowering	1		[1] .86	Sunny.
Do	77	2:30– 3:30	1.6	473.0	451.0	356.7	do	1		[1] .79	Do.
Jan. 23	70	1:34– 2:35	1.5	366.0	367.3	199.5	do	2	4 minutes	.54	Do.
Do	68	2:35– 3:35	1.6	346.1	412.2	213.0	do	2	1 hour	.52	Do.
Jan. 24	71	9:20–10:21	1.6	468.7	376.8	216.6	do	3	1 day	.57	Do.
Do	70	11:11–12:11	1.6	380.4	468.2	273.6	do	3	do	.58	Do.
Do	73	12:11– 1:11	2.0	360.7	440.7	293.1	do	3	do	.67	Do.
Jan. 25	07	1:16– 2:15	1.6	280.6	268.5	182.6	do	4	2 days	.68	Do.
Do	67	2:15– 3:17	1.8	262.6	355.4	186.5	do	4	do	.52	Do.
Jan. 26	68	9:53–10:53	1.4	364.2	326.9	216.2	do	5	3 days	.66	Do.
Feb. 4	73	1:26– 2:24	1.8	372.3	307.2	155.4	Milk	14	12 days	.51	Do.
Feb. 8	68	2:13– 3:11	1.6	339.8	375.7	199.8	Soft dough	18	16 days	.53	Do.
Feb. 15	75	1:50– 2:49	1.4	516.7	340.9	165.9	Hard dough	25	23 days	.49	Cloudy.
Feb. 19	70	2:47– 3:46	.9	618.2	122.0	64.8	do	29	27 days	.53	Do.
Feb. 22	73	1:25– 2:24	1.9	311.6	231.7	103.3	Ripe	32	30 days	.46	Sunny.
SET NO. 4											
Jan. 28	67	9:27–10:26	.9	581.0	121.8	122.9	Early milk	9		[2] 1.01	Cloudy.
Do	74	1:04– 2:04	1.4	503.5	221.4	116.1	do	9	4 minutes	.52	Do.
Do	73	2:04– 3:04	1.5	480.7	222.2	117.8	do	9	64 minutes	.53	Do.
Jan. 29	73	8:17– 9:16	1.1	579.9	102.8	66.8	Milk	10	1 day	.65	Do.
Do	68	9:16–10:16	.9	606.9	375.0	67.9	do	10	do	.18	Do.
Do	67	1:27– 2:28	.8	562.4	166.5	116.1	do	10	do	.70	Do.
Do	66	2:28– 3:28	.7	549.9	132.6	76.8	do	10	do	.58	Do.
Jan. 30	67	8:31– 9:30	1.0	536.9	124.3	100.0	do	11	2 days	.80	Do.
Do	68	9:30–10:29	1.1	560.2	161.2	123.2	do	11	do	.76	Do.
Do	73	1:22– 2:21	1.2	634.3	226.9	124.3	do	11	do	.55	Do.
Do	73	2:21– 3:20	1.2	639.4	227.4	125.3	do	11	do	.55	Do.
Feb. 4	73	2:38– 3:37	1.5	402.5	245.1	157.4	Soft dough	16	7 days	.64	Sunny.
Feb. 8	68	3:16– 4:14	1.5	357.3	302.3	191.0	do	20	11 days	.63	Do.
Feb. 15	75	2:54– 4:53	1.4	500.1	246.0	125.5	Hard dough	27	18 days	.51	Cloudy.
Feb. 19	71	3:56– 4:55	.9	641.4	64.9	37.8	do	31	22 days	.58	Do.
Feb. 22	72	2:29– 3:29	2.0	300.8	103.3	28.3	Ripe	34	25 days	.27	Sunny.
SET NO. 5											
Jan. 31	66	8:32– 9:30	.9	553.7	234.8	229.3	Early milk	10		[1] .98	Do.
Do	70	9:36–10:36	1.3	555.6	295.8	199.1	do	10	6 minutes	.67	Do.
Do	70	10:36–11:37	1.6	527.4	406.4	250.7	do	10	66 minutes	.62	Do.
Do	72	11:37–12:35	2.0	450.7	393.4	198.7	do	[illegible]	2 hours	.51	Do.

Feb. 1	68	9:00- 9:59	1.1	554.4	477.9	275.2	do		1 day	.58	Do.
Do	72	11:00-11:59	.9	493.2	522.4	328.5	do	11	do	.63	Do.
Do	72	12:00-12:58	1.9	437.2	498.2	307.3	do	11	do	.62	Do.
Do	72	2:00- 3:00	1.9	364.6	407.1	237.8	do	11	do	.58	Do.
Feb. 2	70	8:00- 8:59	1.2	552.1	203.7	158.4	Milk	12	2 days	.78	Do.
Do	73	10:00-10:58	2.1	435.6	442.3	273.5	do	12	do	.62	Do.
Do	70	1:00- 1:59	1.4	379.1	336.9	219.8	do	12	do	.65	Cloudy.
Do	73	3:00- 4:00	1.3	498.4	403.4	216.7	do	12	do	.54	Do.
Feb. 5	70	9:00- 9:58	1.0	548.2	217.3	123.4	do	15	5 days	.57	Do.
Do	73	9:58-10:57	1.7	458.3	301.9	173.2	do	15	do	.57	Do.
Do	70	3:37- 4:35	1.1	458.3	205.3	141.3	do	15	do	.69	Do.
Do	70	4:35- 5:33	.8	599.5	175.0	101.2	do	15	do	.58	Do.
Feb. 9	68	10:23-11:21	1.0	472.8	203.6	111.0	Soft dough	19	9 days	.55	Do.
Feb. 16	75	11:56-12:53	2.2	276.5	464.7	243.2	Hard dough	26	16 days	.52	Sunny.
Feb. 20	67	9:24-10:23	.9	518.0	140.6	72.6	do	30	20 days	.52	Cloudy.
Feb. 23	73	12:37- 1:36	1.9	389.5	273.6	140.0	Ripe	33	23 days	.51	Sunny.
Set No. 6											
Feb. 6	67	8:44- 9:43	.8	492.2	332.9	277.8	Milk	14		[2].83	Do.
Do	70	9:50-10:49	1.3	458.5	521.4	323.6	do	14	5 minutes	.62	Do.
Do	67	1:42- 2:40	.9	384.5	332.1	204.3	do	14	4 hours	.62	Do.
Do	70	2:40- 3:38	1.2	426.4	505.4	296.8	do	14	5 hours	.59	Do.
Feb. 7	72	8:45- 9:43	1.0	690.5	164.2	123.2	do	15	1 day	.75	Cloudy.
Do	73	9:43-10:44	1.3	629.9	198.1	141.5	do	15	do	.71	Do.
Do	75	1:45- 2:43	1.7	449.9	297.3	189.6	do	15	do	.64	Do.
Do	74	2:43- 3:43	1.2	518.5	268.8	164.5	do	15	do	.61	Do.
Feb. 9	73	1:03- 2:02	1.0	586.3	270.8	178.2	Soft dough	17	3 days	.66	Do
Do	75	2:03- 3:01	1.2	568.2	350.4	218.9	do	17	do	.62	Sunny.
Do	73	3:01- 4:00	1.9	403.2	397.6	265.6	do	17	do	.67	Do.
Feb. 16	75	1:00- 1:59	2.3	268.5	334.5	226.9	Hard dough	24	10 days	.68	Do.
Feb. 20	68	10:30-11:29	1.1	535.5	131.8	136.8	do	28	12 days	1.04	Cloudy.
Feb. 23	78	1:41- 2:41	2.2	375.9	372.2	240.0	do	31	15 days	.64	Do.
Feb. 26	71	1:45- 2:45	1.3	399.5	166.8	103.7	Ripe	34	18 days	.64	Do.
Set No. 7											
Feb. 12	67	9:18-10:16	.8	607.7	39.8	46.2	Soft dough	20		[2]1.16	Do.
Do	70	10:17-11:21	1.5	446.7	251.4	199.0	do	20	2 minutes	.79	Do.
Do	73	3:05- 4:03	1.4	426.6	187.6	137.5	do	20	5 hours	.73	Do.
Do	72	4:03- 5:02	1.3	451.5	192.4	133.1	do	20	6 hours	.69	Do.
Feb. 13	72	2:45- 3:43	2.4	245.0	464.2	295.6	do	21	1 day	.64	Sunny.
Do	68	3:43- 4:43	1.7	253.5	367.4	244.5	do	21	do	.67	Do.
Do	70	4:43- 5:42	1.1	450.3	219.1	151.7	do	21	do	.69	Do.
Feb. 14	66	8:05- 9:04	.7	619.3	174.3	118.4	Hard dough	22	2 days	.68	Do.
Do	70	9:04-10:03	1.4	530.3	351.8	225.1	do	22	do	.64	Do.
Feb. 16	75	2:05- 3:04	3.8	264.5	409.1	252.4	do	24	4 days	.62	Do.
Do	75	3:04- 4:03	1.7	338.3	346.3	193.3	do	24	do	.56	Do.
Feb. 20	65	3:25- 4:25	1.0	468.7	217.2	120.7	do	28	8 days	.56	Cloudy.
Do	68	4:25- 4:55	1.2	497.6	171.6	124.4	do	28	do	.72	Do.
Feb. 23	73	2:46- 3:45	2.2	369.9	265.0	140.8	do	31	11 days	.53	Do.
Do	71	2:50- 3:51	1.4	348.4	165.6	83.4	Ripe	34	14 days	.50	Do.

[1] All experiments were conducted during daylight hours.
[2] Initial ratio.

Figure 5 shows that the amount of transpiration rose rapidly to a maximum during the period of 1 to 3 days after flowering, following which there was a relatively sudden decline until about the eighth day after flowering. The rate of transpiration then rose and maintained approximately a constant level until the eighteenth day. The rate then declined until the twenty-first day, when it again increased to a second marked maximum approximately 26 days after blooming. During the next 3 days the rate declined to the second lowest point for the season, then increased during the next 3-day period, and again declined to the minimum.

The first maximum is in accordance with the observations of Vasilyev (9) that the maximum transpiration by the awns of Beloturka

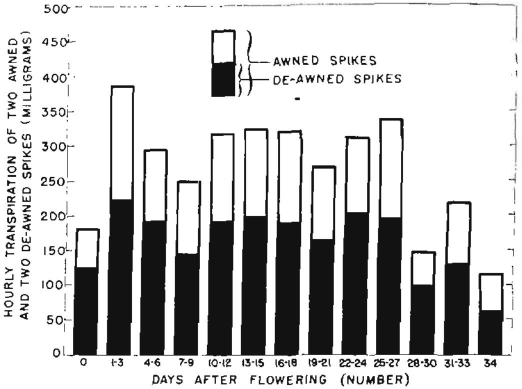

FIGURE 5.—The total transpiration of awned and de-awned heads of wheat at 3-day intervals from flowering to maturity. The transpiration of the awned heads is represented by the total height of the column and includes both the light and shaded portions; the transpiration of the two de-awned heads is represented by the height of the shaded portion of the column.

wheat was at the time of flowering. That portion of the diagram which represents the rate of transpiration from the eighth to the twenty-ninth day after flowering corresponds to the curve reported by Perlitius (6) for spring wheat. For winter wheat, Perlitius obtained a double curve with the maxima occurring at the onset of fruit formation and just preceding the milk stage. Since he made determinations at only six periods between heading and maturity, he did not obtain sufficient data to indicate whether other fluctuations occurred before or after periods of observation.

In the present experiments it is clearly indicated that the variations in the rate of transpiration of the heads were not correlated with the atmospheric conditions of the environment as shown by the rate of evaporation from the porous-cup atmometer. It also seems evident

that there was no correlation between variations in light conditions and variation in the transpiration of the heads. The curve of transpiration of the heads during their development and maturity show two very evident maxima. It seems evident that the heads of wheat at flowering and at the approach of maturity of the grain show marked maxima in the rate of transpiration. The significance of this change of the transpiration rate in the metabolism of the plant is not known.

SUMMARY

The effects of de-awning upon the rate of transpiration of the heads of Pusa 52 × Federation wheat were studied in the greenhouse in 1935–36 and 1936–37. During the first year fifty-five 1-hour determinations were made, and the technique for the following year was developed. During the second year one hundred and eight 1-hour determinations were made.

The rate of transpiration of the awns was determined by the Freeman method, which was modified and improved to meet the specific requirements of the experiment. By this method the net transpiration of the awned and de-awned heads was determined quantitatively. The amount of transpiration attributable to the awns was thus obtained.

The rate of transpiration of the awned and de-awned heads was determined at various times from just before flowering until the head had fully matured. The average ratio of the amount of water transpired from the seven sets of intact heads that later were de-awned to the seven sets of heads that served as controls during the experiments was 0.976. In 108 experiments made as early as 2 minutes after de-awning and as late as 34 days thereafter, and from the early flowering stage of the heads to complete ripeness of the grain, the average ratio of the amount of water transpired from the de-awned to the awned heads was 0.61. On a percentage basis the de-awned heads transpired 38.9 percent less than did the awned heads.

The curves of transpiration of the awned and de-awned heads paralleled each other throughout the experiments from flowering to maturity. These curves showed a maximum rate of transpiration of the heads at flowering and another as the maturity of the head was approached. These variations in the rate of transpiration seemed to be independent of the environmental conditions as indicated by evaporation from a porous-cup atmometer, but the cause of these variations is not known.

The rate of transpiration from the heads of wheat is evidently decreased by de-awning, but the significance of transpiration from these parts in the metabolism of the plant is unknown.

Although the awns of wheat are active in the transpiration of the head, they do not ordinarily transpire more than 1 to 5 percent of the total amount of water lost from the plant.

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A DEVELOPMENTAL ANALYSIS OF KOHLRABI AND CABBAGE STEMS [1]

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INTRODUCTION

Many horticultural and agronomic problems are concerned, either directly or indirectly, with the subject of the relative growth rates of the cells, tissues, and organs of plants. The rate of cell division in relation to rate of enlargement is a fundamental study, which when investigated more fully, should throw light on many problems concerned with the growth of economic plants. Certain physiological and nutrititional problems would doubtless be more clearly understood if more were known of the relation between cell division and enlargement and organ or body size. This field of study, in its application to agricultural research, has evidently been little investigated.

Little is now known of the relationship between genetic constitution and cell and tissue activity in any of the economic plants, except in very general terms. Such genetic interrelationships of cells and tissues within a plant organ are of primary concern in any detailed histological study of the growth phenomena in plants.

Recent studies on the relation between cell size and organ size have been concerned principally with fruits. Sinnott (*4*),[3] however, in a study of *Acer* petioles presented results showing that organ size was to some extent directly proportional to cell size in this plant part. He also reviewed several of the earlier investigations on this subject, which show considerable inconsistency especially in general conclusions. Recent morphogenetic studies with fruits, such as those of Houghtaling (*1*), Sinnott (*6*), and Tukey (*7*), suggest the problem of similar growth phenomena in stems and other organs.

The study here reported was made to determine the relation between cell size and number and stem and tissue sizes in two plants closely related genetically but greatly different in stem volume, the cabbage (*Brassica oleracea* var. *capitata L.*) and the kohlrabi (*B. oleracea* var *caulo-rapa* DC). These plants are both horticultural crops and they are of economic importance largely because of altogether different morphological developments. The terminal bud becomes the economically important or edible part of the cabbage plant; the enlarged stem becomes the edible part of the kohlrabi. The two plants are similar in their seedling stage but can be distinguished by leaf characters beyond that stage.

Based on a genetic study of cabbage and kohlrabi stems, Pease (*3*) concludes that the enlarged stem of the kohlrabi is determined by three multiple factors, two of which are major, the other, a minor or modify-

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[2] The writer is grateful to Dr. E. W. Sinnott, of Columbia University, for many helpful suggestions throughout the course of this investigation and to the staff of the Department of Botany, Columbia University, for the use of its greenhouse and laboratory facilities.

[3] Italic numbers in parentheses refer to Literature Cited, p. 470.

ing one. He presents no data, however, on the morphogenetic relationships of these plants, and there seems to be no literature bearing on this specific subject.

MATERIALS AND METHODS

Plants of the White Vienna variety of kohlrabi and the Copenhagen Market cabbage were used in this study. They were grown in the greenhouses of Columbia University during the fall and winter of 1939–40. Throughout most of the growing period it was necessary to lengthen days to 12 to 14 hours by the use of 500-watt lights in order to secure satisfactory thickening of the kohlrabi stem and to prevent excessive etiolation.

It is essential in such studies as these that the data be secured from comparable positions on the plant throughout growth. Under the conditions of this study, the main axes of both plants grew in height at about the same rate and reached about the same height when the rate of growth in height decreased sharply. This decrease in rate of growth indicated the time of beginning of rapid stem enlargement of the kohlrabi and relatively slow stem enlargement but rapid terminal bud development of the cabbage. The first specimens were collected at this stage of development. After considerable preliminary examination, it was found that a position about 20 mm. from the apex was most satisfactory for stem measurements in both plants throughout their development. Thereafter, studies of the growth relationships were made at very nearly that position, which was at about the center of the mature enlarged kohlrabi stem. The data presented were taken from a study of 121 kohlrabi and 48 cabbage plants. The diameter of stem of kohlrabi ranged from 1 mm. to 90 mm., the cabbage stem diameter ranged from 1 mm. to about 12 mm. All the material represented the stages from shortly after differentiation of primary tissues to approximate maturity of the stems. Because of the apparently unavoidable variation in individual plants, it seems necessary in such studies to use a large number of specimens. Uniform environmental factors are also essential.

The plant material was killed, embedded in paraffin, and cut into transverse and longitudinal sections for certain phases of the study. Most of the investigation, however, was carried out by the use of freehand sections stained with Delafield's haematoxylin. The sections were then temporarily preserved in glycerin for examination and drawing. This latter method proved much more satisfactory, since a large number of specimens could be examined, and also because of the lack of any distortion of cells or cell walls.

The transverse plane was used in determining cell size and tissue size. Since the cells were nearly isodiametric the volume of the cells could be compared with the volume of the tissues or the stem in such a structure as the enlarged kohlrabi. A more accurate method, however, seemed to be to compare the cell diameters with the tissue and stem diameters, since the growth relations in the cabbage and kohlrabi could in this way be compared directly.

Measurements were made of the cell, tissue, and stem diameters by the use of a microprojector. These measurements were made at no less than three levels within 2 to 3 mm. of each other and averaged. Cell measurements were made in the following manner: 20 to 30 cells

in each tissue to be used were drawn under the projector and then 20 of them in each section were measured in two directions. The average of these cell diameters, carefully selected as typical, seemed reliable for this purpose. There was some variation in apparent cell sizes throughout the growth, and those measured represented the average maximum sizes shown.

The development of cell, tissue, and organ size is an exponential process; hence it is more correct to plot the growth values logarithmically. In this study, most of the plotting was done by placing the actual values on double logarithmic paper, but it was found that very wide ranges of values could be most clearly presented by the use of logarithms of actual values.

RESULTS

GENERAL STRUCTURE

The kohlrabi and cabbage stems were evidently similar in anatomy during the early stages of development. The cells within 12 mm. of the terminal meristem were almost entirely responsible for the growth in height of the plant. Those closer than 5 to 7 mm. divided rapidly; those from about 7 to 12 mm. elongated considerably. The region of differentiation seemed to lie between 12 and 16 mm. from the tip. As previously mentioned, the growth concerned in diameter relationships could best be measured at a position about 20 mm. from the tip. At 15 to 20 mm. from the terminal meristem, the pith in both plants made up 70 to 90 percent of the total stem diameter up to a diameter of 4 to 5 mm. The vascular bundles were arranged around the outside of the pith. An active cambium could be readily detected in the bundles, and in most stems there was a less active interfascicular one. The cortex, like the pith, was composed of thin-walled parenchyma during its early development. Later, collenchyma developed in the outer portion, and at near maturity, especially in the kohlrabi, an active periderm was formed in the outermost layers. The anatomy of the kohlrabi stem has been described in some detail by Vöchting (*8*), and it is not necessary to do so here. The writer's observations agreed with Vöchting's in all details noted.

The most striking difference between the structure of the cabbage and kohlrabi stems occured at a stem diameter of 5 to 6 mm. At this stage an inner meristematic region developed in the center of the kohlrabi pith. Shortly after this development, four to six small vascular bundles were laid down in a circle about the group of rapidly dividing central pith cells as seen in transverse sections. As growth continued, these bundles could be seen traversing the pith in all directions, as described by Vöchting (*8*). Rarely were they directly associated with the larger stem bundles already mentioned. Neither this inner meristematic zone nor the medullary bundles were ever found in several other closely related plants examined, including the cabbage, cauliflower, broccoli, and brussels sprouts. In the kohlrabi they first appeared as more or less irregular strands of meristematic cells extending through the central pith. Shortly after their appearance or about the time the ring of medullary bundles was formed, the rapidly dividing cells made up a zone about 1 mm. in diameter as seen in cross section. The zone of cell multiplication enlarged toward the periphery of the pith as growth of the plant continued. At maturity it extended to

within 1 to 2 mm. of the periphery of the pith and thus made up mo
of the volume of the pith zone. Meristematic activity here seeme
always to precede the formation of the pith bundles.

The total pith diameter made up about 90 percent of the kohlra
and 80 percent of the cabbage stem at maturity. The edible portic
of the kohlrabi was, therefore, largely of pith tissue. The bundles ar
cortex made up the remaining volume in about equal proportion
This study was mainly concerned with the pith, although certa
relationships are given for other tissues.

GROWTH RELATIONS IN PITH OF KOHLRABI AND CABBAGE

A very definite relationship could be established between cell si
and tissue size at several regions in the pith of the kohlrabi and cabbag
stems by careful selection of sections at comparable positions on eac
plant. It is sometimes desirable to establish volume rather tha
diameter relationships, but here, because of the decreasing cell size a
the apex is approached, it was felt that simple diameter relations a
the given position (about 20 mm. from the tip) would be most accura
and comparable. The growth of the cabbage pith seemed to be mostl
in width or diameter, since there was little or no increase in length a
the position measured, and there was apparently only periclinal an
transverse division of cells. Since the largest cells of the pith of th
kohlrabi stem were nearly isodiametric and the shape of the enti
stem was similar to a sphere, volume relations could be establishe
on that basis if an accurate measure of average cell volume could b
made. No method was known, however, by which an accurate volum
could be obtained for cells which varied in size in different parts o
both the pith and cortex and in shape during division and enlargemen
Certain linear relationships, based on diamter at a definite positio
could be established, however. The results from a large number o
measurements show a somewhat simple growth pattern.

In order to secure a measure of the relative amount of cell divisio
and cell enlargement during the growth of the pith, several types o
measurements were made. Some of the relationships are shown i
figure 1. The cell diameters of the central pith zone and the periphera
pith region were plotted against the total pith diameters on a doubl
logarithmic grid for both kohlrabi and cabbage. The changes i
relative size of the cells and the tissue can then be easily determined b
measuring the ratio between the cell size and pith size at numerou
stages during increase in size of the pith. This relationship can b
expressed by use of the value of k,[4] the constant of relative growth a
used by Huxley (*2*). If the change in relative size of cell and pith i
constant and the values at different sizes are plotted against each othe
logarithmically, a straight line will be formed. The slope of this lin
which can be measured by the value of the constant, k, denotes th
relationship between the two size variables. For example, if cell siz
is increasing at the same rate as organ size, then the line slopes upwar
at an angle of 45° and the value of k is equal to 1.0. If the cell siz
increases more slowly than the organ size, k is less than 1.0, and it

[4] From Huxley's formula for measuring the relationship between the magnitudes of two variables. Whe
x is the value of the organ and y that of the varying part, the relation between them is $\log y = \log b + k \log x$
where b and k are constants. b indicates the value of y when $x=1$, and is of no importance here. The valu
of k denotes the relation between the two variables. This simply means that if two variables which follo
this formula are plotted against each other on a double logarithmic grid their values will fall in straight lines

value measures the relation between the two variables. If cell size is not increasing as rapidly as organ size, the difference in the relative value between them must be due to cell division. The relative amount of cell division to cell enlargement can then be calculated from the value of the relative growth constant or the relation between these two variables, or *k*. These relationships are, of course, independent of time and of absolute rate of growth.

As may be noted (fig. 1, *a*, *b*), growth in the central pith zone in both the kohlrabi and the cabbage was due to both cell division and cell enlargement. Cell division was responsible for most of it, however, up to a total pith diameter of about 2.2 mm., since up to this point the relative growth constant *k* is equal to 0.2 to 0.3. In other

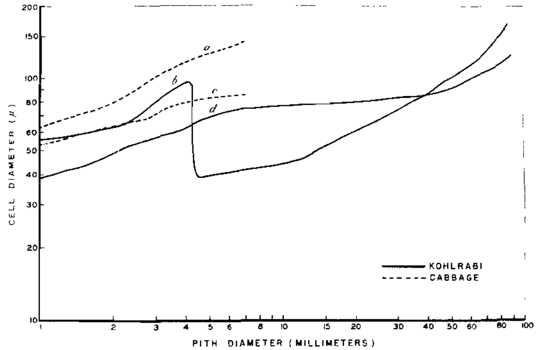

FIGURE 1.—Cell diameter plotted logarithmically against pith diameter during development of the kohlrabi and cabbage stems: *a*, *b*, Central pith cells; *c*, *d*, peripheral pith cells. The sharp break in *b* at a pith diameter of 4 to 5 mm. shows the cell relationships there at the time of development of the inner meristematic zone.

words, 20 to 30 percent of the growth was due to cell enlargement, the remainder, to cell division. At the pith diameter of about 2.5 mm., the kohlrabi central pith showed a rather sharp increase in relative rate of cell enlargement which extended to a total diameter of about 4.2 mm. At the 4.2-mm. diameter, cell enlargement was evidently responsible for about 75 percent of the growth. Cell division rate decreased at about the same stage in the cabbage, but up nearly to maturity it was responsible for about 50 percent of the increase in diameter of the pith (fig. 1). There was no sharp inflection point or position where cell division stopped and cell enlargement began.

The central meristematic zone already described appeared in the kohlrabi pith when it was between 4.0 and 4.5 mm. in diameter. With its appearance there was a sharp decrease in the average cell diameter in this region, as shown in figure 1, *b*. Thereafter, up to a

pith diameter of about 12 mm., cell division was responsible for abou 80 percent of the growth in relation to the tissue as a whole. A growth continued, cell division was accompanied by more and mor enlargement after each division, until at between 70 and 90 mm. i total diameter, the inner pith cells were enlarging at the same rat as the pith diameter, and $k=1.0$. Cell division evidently had the ceased, and the growth was due entirely to cell enlargement here.

The peripheral pith cells of both the kohlrabi and the cabbage wer measured in successive stages of stem development in order to com pare their relative rate of cell division and enlargement with that c the central cells just described. The peripheral pith cells measure were those from about the fifth to tenth cell removed from and insid the vascular bundles. The same general relationships appeared i both the kohlrabi and the cabbage up to the time of development c the inner meristematic pith zone in the kohlrabi, or to a pith diamete of about 4.5 mm. (fig. 1, *c*, *d*). Up to this point in the kohlrab and throughout the growth of the cabbage, the relative rate of ce division to cell enlargement was greater in the peripheral zone tha in the central one. In other words, growth due to proportionatel more cell enlargement occurred first in the center of the pith. Fron the center toward the periphery, there was a definite gradient i this type of growth which could be observed readily by microscopi examination at increasing size of the tissue. The difference in rate o cell division in proportion to cell enlargement in the two zones is wel illustrated by the constantly greater value of k (fig. 1) for the centra pith of the cabbage and during the early growth of the kohlrabi.

The general relationship just described was upset somewhat by th development of the central meristematic zone in the kohlrabi pith Evidently, 60 to 70 percent of the growth at the periphery of th kohlrabi pith was due to cell division until a pith diameter of approxi mately 6 mm. was reached. After that size was attained there wa very little change in cell size or in relative rate of cell division unti rapid cell enlargement took place soon after the period of rapid en largement of cells in the central pith just before maturity (fig. 1) The peripheral pith cells then remained larger than the inner one throughout most of the period of enlargement of the kohlrabi stem This is evidently a very unusual situation in plant tissues, however In fact, examination of the cells included in the central meristemati zone showed that within it the largest cells were usually found nearest the center.

RELATIVE NUMBER OF CELLS

The regression line in figure 1 shows the relative changes in cell size at increasing kohlrabi pith size, and from these one may see where increase in cell number must be taking place most rapidly in propor- tion to pith size, as already described. Nevertheless, in order to clarify the relative changes in cell number, the actual number of cells can be plotted against the pith volume of the enlarged stem or "knob" of the kohlrabi (fig. 2). Because of the extreme range in number of cells, as well as in pith volume, it was more convenient to plot the logarithms of the values rather than the actual values. The measure- ments were made, their logarithms located on the graph paper, and then regression lines drawn through them as in the other figures.

The number of cells at any stage in the growth of the kohlrabi pith can be determined approximately by dividing the average volume of the cells into the pith volume. To determine the average volume of the pith cells, three regions were used: The center of the pith, the outermost part of the developing meristematic pith region (after it appeared), and the peripheral pith zone. The average cell volume for each was calculated on the basis of a sphere. Then a weighted average cell volume was calculated, based on the respective volumes of the inner and peripheral pith zones. The cell volume thus determined was then divided into the total pith volume, based also on a sphere. The results from 121 plants used in these determinations fit into a linear regression line (fig. 2). The logarithms of the actual values are shown, since cell number and organ volnme are more nearly logarithmic or multiple, than additive relationships. Because of the

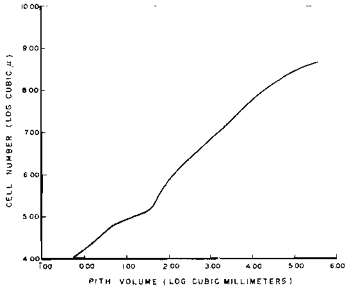

FIGURE 2.—Logarithm of cell number plotted against logarithm of pith volume in the kohlrabi stem during development of the edible portion. The sharp increase in relative cell number at a pith volume of about 1.7 marks the beginning of the rapid cell division in the inner pith.

method of calculation, these results are not as accurate as those presented in figure 1. Nevertheless, the line shows that cell number increases rapidly in the early stages of growth and increases more slowly when the pith is between 2.5 and 4 mm. in diameter. In the later stages, the advent of the central pith cell divisions was indicated by the sharp increase in cell number. The rate of increase was thereafter fairly constant until near maturity, when there was a gradual reduction in rate of increase in number of cells.

The changes in cell number in the cabbage were calculated on the basis of the diameter, that is, the number of cells across the pith in proportion to the diameter of the pith and to that of the stem (fig. 3). The line in this figure which compares the cell number to the diameter of the pith is more accurate, since the pith was growing more rapidly than the stem as a whole. If they had been growing at the same relative rate, either method of comparison would have been satisfactory. As may be noted (fig. 3), the number of cells increased at a fairly constant rate throughout the growth of the stem.

CELL RELATIONSHIPS IN THE CORTEX

The relationship between the size of the cortical cells and the si either of the organ as a whole or of the tissue itself was so variab that a graphic presentation of the results would not be very reliabl The cells varied in size considerably in different plants of the sam diameter and also at different positions in the same plant. Neve theless, measurements of cell and tissue diameters were made he with as much accuracy as feasible by the same method as used i the pith.

The cortex increased from a radius of 0.2 to 0.3 mm. when the ste was about 4 mm. in diameter to a radius of 1.5 to 2 mm. when th stem of the kohlrabi was 90 to 100 mm. in diameter. The same rel tionship was shown in the cabbage, but growth of the cabbage ste was limited to 12 to 14 mm. in diameter. There was much less i

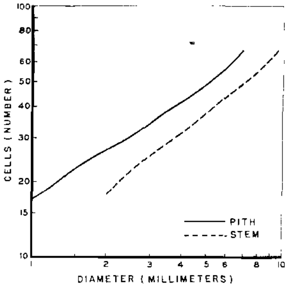

FIGURE 3.—Cell number plotted against pith and stem diameter during th increase in diameter of the cabbage stem. The number of cells represent those in a cross-section or diameter of the pith.

crease in cell size with increase in tissue size than in the pith cells The average diameter of the cortical cells increased from about 30μ to 40μ during the development of the kohlrabi stem. Periclinal an transverse cell division persisted to maturity. The cabbage plants showed the same relationships in proportion to the diameter of th stem.

RELATIVE GROWTH RATE OF PITH AND ORGANS

The pith diameter was plotted against the total stem diameter at increasing sizes of the kohlrabi and cabbage stems in order to determine the relative growth rates of the pith and stem in these plants (fig. 4). Sinnott (*5*) presented results which showed that in the species he studied, the pith increased more rapidly than the remainder of the stem and that in progressively larger stems "the pith assumes an increasingly greater share of the total size and tissues outside it a smaller and smaller one." Since the pith in kohlrabi included 65 to 70 percent of the diameter of the stem when it was 3 to 4 mm. in diameter, and 90 percent of it at maturity, it is evident that the pith

increased in size more rapidly than the body or stem. Nevertheless, the relative value of this difference in the growth rate of the pith and the stem as a whole was not great. As pointed out by Huxley (*2*), comparatively small variations in the value of *k*, when plotted on a logarithmic scale, will give large differences if growth continues over a great range in size.

The value of *k* was determined at several regions along the regression lines (fig. 4) and for the lines as a whole. In all cases, *k* assumed

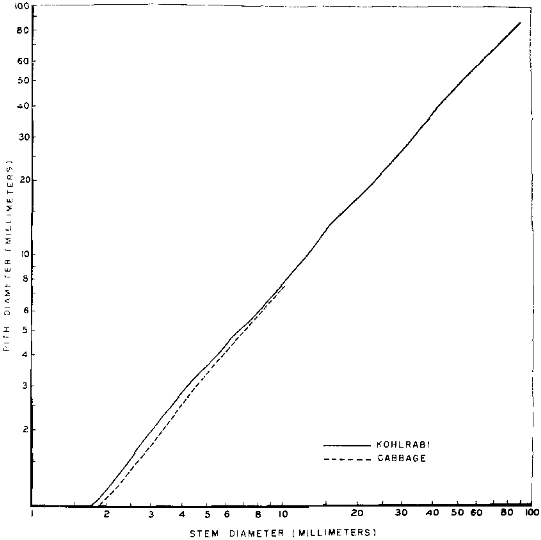

FIGURE 4.—Pith diameter plotted (logarithmically) against total stem diameter of the kohlrabi and cabbage. Both regression lines show *k* values of very nearly 1.1 throughout the growth of both stems.

a value of very nearly 1.1. This means that the pith was growing 1.1 times as rapidly as the stem as a whole. It might be supposed that the development of the central meristematic pith zone in the kohlrabi would increase its rate of growth in relation to the entire stem, since this zone precedes the formation of the "knob" or edible portion of the kohlrabi, which is composed of about 90 percent pith at maturity. This was not the case, however. It is especially significant that the value of *k* remained the same here throughout the growth of the stem in spite of the great increase in rate of cell division at the pith

diameter of about 4.5 mm. It is also significant that the growth constant k is very similar for both the cabbage and the kohlrabi (fig. 4); that is, the regression lines slope upward at the same angle. The difference, insofar as these tissue relationships are concerned, was simply in the size of the stem, not in changes in pith and stem growth relationships. In both plants, therefore, the growth of the pith has the same positive and constant differential growth relationship to organ or stem growth. In other words, the cabbage pith might become as large as that of the kohlrabi without change in its growth relationship to the entire stem; it is just a matter of extent of total growth.

GRAFTING TESTS WITH KOHLRABI AND CABBAGE STEMS

Several grafting tests were made to determine whether it was possible to affect the cell relations of either cabbage or kohlrabi stems by interchange and substitution of plant parts. For instance, it was especially desirable to know whether a cabbage stem would assume any of the characteristics of the kohlrabi if the upper portion of a plant, or that bearing the leaves, was of kohlrabi and the lower portion of the stem was of cabbage, and also whether there would be any transmission of cell and tissue characteristics if an approach graft of the stems of the two plants was made. In this way the cells and tissues of one plant could be caused to grow for a considerable distance along the stems in direct contact with those of the other plant.

It was found that cabbage and kohlrabi stems could be grafted rather easily. Several types of grafts were made at different stages of growth and at different positions on the plants. It is only necessary to state here that in no case did either plant seem to affect the cell or tissue relations in the other. In other words, the cells of each plant retained their own morphogenetic nature regardless of their relationship to cells of another genetic character. These results are, perhaps, not surprising to those who are familiar with stock and scion relations in horticultural material. Nevertheless, they seem worthy of note in view of the recent work on the relation of transfer of certain growth substances to cell activity in plants.

DISCUSSION

The results of studies of kohlrabi and cabbage stem growth are significant because the plants, which are closely related genetically, were found to be similar in certain tissue growth relationships but markedly different in others. The decrease in relative rate of cell division, first in the innermost part of the pith and then gradually toward the periphery, is notable, since the same general trend has been noted in petioles (*4*) and cucurbit fruits (*6*). As in these fruits, there was much increase in cell volume, especially in the kohlrabi pith, even during the period of cell division. The inflection point marking the end of cell division and the period of cell enlargement was not so clearly marked in kohlrabi and cabbage stems, however, as was noted in cucurbit fruits. In general, however, there was a gradual decrease in the relative rate of cell division to cell enlargement as the stems increased in size. Thus these observations and calculations show that in this pith tissue there was more and more cell enlargement after each cell division.

The development of the central meristematic zone in the pith of the ohlrabi seems remarkable, since this zone is the first to show a de- reasing rate of cell division in the early stages. The medullary bundles re evidently not concerned with the initiation of this zone, since rapid ell division begins before these bundles are laid down. It is significant hat as soon as these innermost cells reach a diameter of about 95μ hey begin rapid division. During the growth in size of the pith, the ells farther and farther removed from the center reach that diameter, nd then they start rapid division. The most peripheral pith cells do ot reach 95μ until very late in the development of the stem, and this low increase in size may be the reason why these peripheral pith cells ever become extremely meristematic, as do the more central cells. The basic reason for the early difference in relative rates of cell division o cell enlargement between the central and peripheral pith cells is not lear.

In the cabbage, rate of cell division decreases first in the central ith, then in successively more peripheral cells, as in the early stages of kohlrabi growth. Its activity persists longer in the extreme peri- pheral pith and in the cortex (considering only the primary tissues). These results suggest that perhaps some growth substance or physio- ogical factor associated with the bundles might be responsible for the lelay in maturity of nearby cells. Such a factor would not explain he condition in the pith of the older kohlrabi, however, where the entral meristematic zone develops.

That there is no relationship between cell division and total growth of pith size in proportion to stem size is well illustrated in this study. In spite of the advent of the very meristematic zone in the kohlrabi ith, the same rate of growth relative to the stem as a whole obtains ere as in its earlier growth, and as in the cabbage stem. The morpho- genetic difference between the kohlrabi and the cabbage stems seems o be due largely to the persistence of cell division, accompanied by ell enlargement, to a greater stem size before growth ceases. The lifference is also partly due to the greater size finally attained by the ohlrabi pith cells.

It may be concluded that there are several morphogenetic factors nvolved in the development of size differences in the stems of closely elated plants. Thus, the genetic factors concerned with histological levelopment are responsible for several definite structural differences etween the cabbage and kohlrabi stems. Some of these can be meas- red by a quantitative analysis of structural changes during the ourse of development.

It would seem that a more fundamental solution to many horti- ultural problems could be obtained if more were known of the histo- ogical development of the plants or plant parts concerned. More night be known also about the influence of different practices or reatments on the relative rates of cell division and cell enlargement nd on tissue and organ size relationships.

SUMMARY

In developing kohlrabi and cabbage stems cell diameter in the pith nd cortex was compared with pith and also with stem diameters.

The greater size of the kohlrabi stem was due largely to a greater umber of cells, although some of the difference was evidently due to ell size.

The pith, of which the edible kohlrabi is largely composed, grew a the same rate, relative to the entire stem, throughout its development and at the same relative rate as that of the cabbage pith to its stem

There was no sharp point of demarcation between the cell divisio stage and the cell enlargement stage in the growth of the pith an cortex of these stems.

The innermost cells of the pith increased most rapidly in size, and in the kohlrabi, were the first to become very meristematic agai after a definite size was reached.

By use of grafting tests with kohlrabi and cabbage stems, it wa found that the cells of each plant retained their own morphogeneti nature even though they developed in direct contact with each other

Some factors involved in the histological differences between th stems of the two plants are discussed.

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THE APPARENT DIGESTIBILITY AND NUTRITIVE VALUE OF BEARDLESS WHEATGRASS AT THREE STAGES OF MATURITY [1]

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INTRODUCTION

The digestibility and nutritive value of range grasses in the green, immature stages have not been studied extensively by research workers and there is little information available concerning the relative feeding value of range forage at various stages of maturity.

Some studies have been made concerning the changes in the chemical composition of range plants as maturity advances. However, the chemical composition is not a true index of the nutritive value, partly because of differences in coefficients of apparent digestibility of the various organic nutrients and because of certain other differences such as those relating to palatability.

The study reported in this paper was made to determine the chemical composition and the coefficients of apparent digestibility of the organic nutrients of beardless wheatgrass (*Agropyron inerme* (Scribn. and Smith) Rydb.) at a 3- to 5-inch stage, a 7- to 10-inch stage, and a headed stage of growth. These three stages of development will hereafter be referred to as the early, medium, and late stages, respectively. Beardless wheatgrass is an important native range bunchgrass in the State of Washington and the Pacific Northwest. It is quite similar to bluebunch wheatgrass (*A. spicatum* (Pursh) Scribn. and Smith) except that the former, as the name implies, is awnless, whereas bluebunch wheatgrass is awned or bearded.

REVIEW OF LITERATURE

SEASONAL VARIATION IN CHEMICAL COMPOSITION

Much of the work concerning the seasonal variation in chemical composition has been done with tame grasses. This review includes papers which deal only with range grasses.

McCall (*11*),[3] in studying the chemical changes of bluebunch fescue (*Festuca idahoensis* Elmer) clipped at semimonthly intervals, found the protein content declined from 25.55 percent in the young growing grass to 4.56 percent in the mature grass 4 months later. The amount of crude fiber increased as the plant matured and nitrogen-free extract reached its highest point at maturity. The values for crude fat showed a tendency to decline as maturity advanced and crude ash values showed the opposite trend. The highest percentages of calcium and phosphorus were found in the young grass. The phosphorus

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[2] The author wishes to express his appreciation for helpful suggestions and criticisms to Jerry Sotola and H. Hackedorn, of the Washington Agricultural Experiment Station, and to E. H. Steffen, of the college faculty.

[3] Italic numbers in parentheses refer to Literature Cited, p. 478.

content declined more rapidly than the calcium content as maturity advanced.

Seasonal changes in the chemical composition of blue grama (*Bouteloua gracilis*), hairy grama (*Bouteloua hirsua*), and curly mesquite (*Hilaria belangeri*) were studied by Stanley and Hodgson (*17*). Amounts of moisture, crude protein, and phosphorus were high in the young plants and decreased as the plants matured. The percentages of total ash and the ether extract content showed no definite trends. Amounts of nitrogen-free extract and crude fiber increased as maturity advanced. The percentage of calcium was highest when the plants were young.

Gordon and Sampson (*7*), working with graminaceous, grasslike, and broadleaved herbaceous species of foothill range plants, found a rather orderly decline in the percentage of crude protein, a silica-free ash, calcium, phosphorus, and potassium from early growth to plant maturity. The percentage of crude fiber increased as maturity advanced. The most rapid changes in composition occurred from the early leaf stage to the full-bloom period.

Hart, Guilbert, and Goss (*8*) found that the dry matter of California range forages varies from that of a protein-rich concentrate during the early vegetative stages to that of a poor roughage during the drought period.

The carotene content of black grama (*Bouteloua eriopoda* (Torr.) Torr.) and mesa dropseed grass (*Sporobolus flexuosus* (Thurb.) Rydb.) was found by Watkins (*18*) to be highest during the early growing season.

Smith and Stanley (*14*) tested blue grama grass (*Bouteloua gracilis*) at three stages for vitamin A by the rat-growth method, and found that the young grass was a very potent source of vitamin A. There was only about one-half as much vitamin A in the grass at maturity, and after weathering had taken place the amount was negligible.

According to Atkeson and his coworkers (*4*) little bluestem (*Andropogon scoparius*), big bluestem, (*Andropogon furcatus*), and buffalo grass (*Buchloë dactyloides*) have their highest carotene content in June. When growth ceases the carotene content quickly decreases.

DIGESTION STUDIES

Kennedy and Dinsmore (*9*) conducted one of the early digestion experiments with green range forages. They found the crude protein to be 68.0 and 63.9 percent digestible for western bromegrass (*Bromus marginatus*) and native bluegrass (*Poa sandbergii*) with nutritive ratios of 1:8.5, and 1:8.7, respectively. The grasses were headed when fed.

In an early experiment with a Guernsey cow fed mixed pasture grass, Armsby and Caldwell (*2*) found that the digestibility of all the nutrients decreased materially as the forage matured.

Woodman, Blunt, and Stewart (*21*, *22*) state that early grass is rich in digestible protein and is, in effect, a "watered" protein concentrate. They found that crude fiber in young pasture grass was about 80 percent digestible. The organic matter of pasture grass was about 10 percent less digestible when grazed in June than when grazed a month earlier, according to Woodman and coworkers (*23*).

The dry matter in fresh young grass was 74.4 percent and crude fiber 80.4 percent digestible as determined by tests with two sheep by

Vatson and Ferguson (*20*). Newlander and Jones (*13*) found the ry matter, protein, fiber, nitrogen-free extract, and ether extract of resh young grass to be 71.0, 76.5, 74.4, 77.9, and 40.0 percent digestible s determined with dairy cows.

Christensen and Hopper (*5*) found that the digestibility of mature rairie hay consisting of 50 to 75 percent of western needlegrass *Stipa comata*) was considerably higher when cut in July than when ft until October.

McCall[4] studied the digestibility of mature bluebunch wheatgrass *Agropyron spicatum*) which was gathered in October The coefficients of digestibility as determined with six sheep were 36.0 percent or the dry matter, 50.6 percent for the crude fiber, 38.8 percent for itrogen-free exact, and 32.0 percent for crude fat. The digestion oefficients for crude protein were negative and there was an average egative nitrogen balance of 17 gm. per sheep for the 10-day period. On an 88.76-percent dry-matter basis, the bluebunch wheatgrass conained 34.23 percent total digestible nutrients.

Crampton and Forshaw (*6*), working with rabbits, studied the ntraseasonal changes in the nutritive value of pasture herbage clipings consisting of Kentucky bluegrass, redtop, and wild white clover, ut at 10-day intervals, and found "a progressive decline in growthromoting value and digestibility of herbage from spring until midummer and a complete recovery in both respects in the fall-growth aterial." There was a gradual decrease in digestibility of dry atter, nitrogen, cellulose, and nitrogen-free extract as the season dvanced, followed by a complete recovery in the fall months.

EXPERIMENTAL METHODS AND PROCEDURE

Crossbred range yearling sheep from Lincoln × Merino ewes and Hampshire rams were used. The individual average weights ranged om 38 to 53 kg. (84 to 117 pounds). Each feeding period consisted f 10 days and was preceded by a 3-day preliminary period. A escription of the metabolism cages and methods employed has been iven by Sotola (*15*).

Grass was clipped daily with sheep shears from native stands near Colton, Wash., of almost pure beardless wheatgrass which had never een plowed or cultivated. The bunches were clipped as close to the round as was possible without injuring the crown of the plants. The grass was fed green and the sheep given all they would consume. Five grams of salt containing 0.02 percent of potassium iodide was fed aily, and water was available at all times.

The trials on the medium and late grasses were conducted in the pring of 1938 and the early stage in the spring of 1939. The trial f the late stage was begun as soon as the grass was well headed and efore it started to become dry. The anther-falling stage occurred hen the late digestion trial was about half over. Analytical proedures as recommended by the Association of Official Agricultural Chemists (*3*) were followed in making the analyses.

[4] McCall, Ralph. Seasonal variation in the chemical composition and digestibility of certain ecies of range grasses. 137 pp., illus. 1932. [Thesis, Wash. State Col.]

CHEMICAL COMPOSITION IN RELATION TO MATURITY

The chemical composition of beardless wheatgrass is given in table 1 on the fresh basis and on the water-free basis. The dry matter of the grass was about 14 percent greater at the late stage than at either of the younger stages. The dry-matter content at the early stage was slightly higher than at the medium stage. This was probably because it was taken from an area which was drier and more rocky than the area from which the grass at the medium stage was clipped. As has been stated, the trials on the two youngest stages were conducted in different seasons, but a study of the rainfall and temperature of the two seasons does not show any appreciable differences in these two factors.

The crude protein percentage decreased materially as the grass matured. There was a slight variation in the crude fat content but the changes were not marked. There was nearly twice as much crude fiber in the grass at the late stage as at either the early or medium stages. This is probably a major factor in the reduction of digestibility as the grass matured. The nitrogen-free extract increased about 5 percent in the late stage, and the difference was slight between the two younger stages. The crude-ash content remained about the same in the younger stages and increased slightly in the late stage on the fresh basis.

TABLE 1.—*Chemical composition of beardless wheatgrass at three stages of maturity*

Basis and state of maturity	Dry matter	Crude protein (N × 6.25)	Crude fat	Crude fiber	N-free extract	Crude ash	Calcium	Phosphorus	P:Ca ratio (P as 1)
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Fresh grass:									
Early	32.49	6.75	1.32	8.04	13.05	3.33	0.14	0.12	1.17
Medium	31.80	5.25	1.30	8.98	12.97	3.30	.11	.09	1.22
Late	45.51	4.07	1.57	15.51	20.80	3.56	.14	.11	1.27
Dry matter:									
Early	----	20.79	4.06	24.75	40.16	10.24	.44	.37	1.19
Medium	----	16.52	4.08	28.24	40.78	10.38	.35	.29	1.21
Late	----	8.95	3.45	34.07	45.71	7.82	.30	.24	1.25

The highest content of calcium and phosphorus in the beardless wheatgrass coincided with the youngest stage and decreased as the grass matured, with a slight widening of the calcium-to-phosphorus ratio. The content of calcium and phosphorus compares favorably with that found by McCall (*11*) for bluebunch fescue at corresponding stages of maturity. The calcium-phosphorus ratio does not equal the approximately 2:1 ratio found in the bones of animals, but lies within the normal limits of the 2:1 and 1:2 calcium-phosphorus rat given by Maynard (*10*).

DIGESTION STUDIES

COEFFICIENTS OF APPARENT DIGESTIBILITY

Table 2 contains the coefficients of apparent digestibility as det nined with individual sheep, as well as the average for each trial.

The coefficients of apparent digestibility of all the organic nutrie lecreased as the grass matured with the exception of the crude

ch was slightly more digestible at the medium stage than at the ly stage. The decline indigestibility was greater between the dium and late stages than between the early and medium stages naturity. The nutrients at the two younger stages resemble those a concentrate rather than a roughage. The fat and crude protein reased most in digestibility as maturity advanced, followed in er by the fiber and nitrogen-free extract.

LE 2.—*Coefficients of apparent digestibility of beardless wheatgrass at three stages of maturity*

age of maturity	Sheep No.	Sex	Dry matter	Crude protein (N × 6.25)	Crude fat	Crude fiber	N-free extract
			Percent	*Percent*	*Percent*	*Percent*	*Percent*
	1	M	72.0	80.0	69.0	77.0	77.0
	3	M	72.0	80.0	61.0	79.0	77.0
	4	M	71.0	78.0	63.0	78.0	74.0
Average			71.7	79.3	64.3	78.0	76.0
	1	F	68.0	75.0	67.0	74.0	74.0
	2	F	68.0	76.0	68.0	76.0	74.0
	3	F	69.0	77.0	67.0	77.0	74.0
um	4	F	69.0	76.0	66.0	75.0	75.0
	5	M	67.0	75.0	63.0	73.0	73.0
	6	F	67.0	77.0	65.0	72.0	73.0
Average			68.0	76.0	66.0	74.5	73.8
	1	F	62.0	64.0	46.0	66.0	67.0
	2	F	64.0	62.0	46.0	70.0	69.0
	3	F	64.0	66.0	48.0	69.0	68.0
	4	F	63.0	65.0	53.0	66.0	68.0
Average			63.2	64.2	48.2	67.8	68.0

DIGESTIBLE NUTRIENTS

'he digestible nutrients and nutritive ratios of fresh beardless atgrass at the three stages of maturity are shown in table 3. hough the digestion coefficients of the various nutrients were er in the late stage than in the immature stages (table 2), the centage of total digestible nutrients of an equal weight of fresh en grass was about 6 percent larger than in either of the other stages i result of the larger percentage of dry matter in the late stage.

'he difference in total digestible nutrients between the two younger es was not marked, although the early stage was slightly higher in stible nutrients than the medium stage, owing to the slightly ier digestion coefficients and the greater dry-matter content. digestible nutrients depend on the dry-matter content as well the digestion coefficients and would vary according to the dry ter when expressed on a basis of fresh grass. When computed on ry-matter basis, the total digestible nutrients were 72.10, 69.85, 63.62 percent for the early, medium, and late stages, respectively, wing that the digestible nutrients in the dry matter decreased as grass matured.

'here was a marked difference in the quality of the feed at the e stages of maturity as evidenced by the protein content and ritive ratio. The digestible protein decreased from 5.34 percent he early stage to 2.61 percent in the late stage. The nutritive o increased from 1:3.39 to 1:10.10 in these same stages. When

put on a 10-percent moisture basis, the percentages of total digesti nutrients of the early stage and the medium stage were 7 and 5 perc higher, respectively, than the average total digestible nutrients foı by Sotola (*16*) for the leaves of first, second, and third cuttings alfalfa hay. The nutritive ratios of the grass were slightly wi than for the alfalfa leaves. The total digestible nutrients for grass at the late stage was about the same as for alfalfa leaves, but nutritive ratio was about 3.5 times as wide.

TABLE 3.—*Digestible nutrients in fresh beardless wheatgrass at three stage: maturity*

Stage of maturity	Total dry matter	Dry *matter*	Crude protein (N × 6.25)	Crude *fat*	Crude *fiber*	N-free *extract*	Total digestible nutrients	Nu tri *rat* 1 to
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Early	32.49	23.41	5.34	0.85	6.27	9.91	23.43	
Medium	31.80	21.70	3.99	.86	6.71	9.59	22.23	
Late	45.51	28.76	2.61	.76	10.50	14.14	28.96	1

CONSUMPTION OF FRESH GRASS AND WEIGHTS AND GAINS OF SHEEP

The daily consumption of fresh grass and the average gains mε by sheep during the 10-day digestion trials are presented in table The gains made on the grass at the three stages of growth decrea: slightly as the grass matured. However, too much emphasis canı be placed on the weight records and gains because of the short per: involved and the weights being based on 1-day weighings. T sheep also had free access to water before being weighed.

The amount of fresh grass consumed daily decreased with advanc maturity. This decrease was quite marked between the medium a late stages.

TABLE 4.—*Daily consumption of fresh grass, average weights, and gains by sheep beardless wheatgrass at three stages of maturity*

Stage of maturity	Average weight of sheep	Average gain by sheep in 10 days	Average daily consumption of fresh grass
	Kilograms	*Kilograms*	*Kilograms*
Early	42.07	1.89	3.10
Medium	43.15	1.78	2.98
Late	46.61	1.36	2.13

CONSUMPTION OF DRY MATTER, DIGESTIBLE NUTRIENTS, AND CALCIUM AND PHOSPHORUS

The average daily consumption of dry matter, digestible nutrien and calcium and phosphorus per 45.36 kg. (100 pounds) body weig are given in table 5.

The consumption of dry matter decreased materially as the grε matured even though the more immature fresh grass had a much high percentage of water, necessitating the handling of more bulk by t sheep. Since the percentage of total digestible nutrients of the d matter decreased as maturity advanced, it follows that the consum

tion of digestible nutrients was less at the advanced stages of maturity. The differences in the dry-matter intake and different planes of nutrition at the three stages of maturity should have no effect on the coefficients of apparent digestibility, as has been shown by Armsby (*1*), Watson and coworkers (*19*), and Woodman and coworkers (*23*).

The larger consumption of fresh grass and dry matter at the immature stages indicates that the young grass is more succulent and palatable to sheep than grass that is more mature. This should result in a larger consumption of digestible nutrients of a higher quality by animals when grazing on the more immature forage than when grazing on mature forage, with a resulting faster rate of increase in weight and growth. That such is the case is shown by the consumption and gain figures in tables 4 and 5.

TABLE 5.—*Average daily consumption of dry matter, digestible nutrients, and calcium and phosphorus per 45.36 kg. (100 pounds) body weight by sheep fed beardless wheatgrass at three stages of maturity*

Stage of maturity	Dry matter consumed daily	Total digestible nutrients in dry matter	Total digestible nutrients consumed daily	Calcium consumed daily	Phosphorus consumed daily
	Grams	*Percent*	*Grams*	*Grams*	*Grams*
Early	1,072	72.10	773	4.72	3.97
Medium	931	69.85	650	3.26	2.70
Late	865	63.62	550	2.60	2.08

The calcium and phosphorus figures show a rather large daily intake of both of these minerals. The consumption is from two to three times greater than the calcium and phosphorus requirements estimated by Mitchell and McClure (*12*) for growing Shropshire sheep.

NITROGEN BALANCE STUDIES

The data of the nitrogen balance studies during the 10-day feeding period are presented in table 6. A positive nitrogen balance was shown for all three trials. The nitrogen retention was greater for the two younger stages of maturity than for the late stage. The variation for nitrogen intake stored for the individual sheep in the last two trials was quite large, being from 10 to 25 percent in the medium stage, and from 3 to 18 percent in the late stage.

TABLE 6.—*Average results of nitrogen metabolism studies with sheep fed beardless wheatgrass at three stages of maturity*

State of maturity	Nitrogen consumed	Nitrogen voided			Nitrogen balance	Intake stored
		Feces	Urine	Total		
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
Early	331	69	217	286	45	13.60
Medium	241	58	140	198	43	17.84
Late	128	46	70	116	12	9.38

SUMMARY AND CONCLUSIONS

A summary of the results of 10-day digestion trials with yearling sheep fed beardless wheatgrass (*Agropyron inerme* (Scribn. and Smith) Rydb.) at an early (3- to 5-inch) stage, a medium (7- to 10-inch) stage, and a late (headed) stage is presented.

The crude protein content decreased as the plant matured while the crude fiber and nitrogen-free extract increased. The crude fat and crude ash showed slight increases.

The coefficients of apparent digestibility of various nutrients decreased as the grass matured. All the nutrients were highly digestible in the youngest stages. The crude protein was 79 percent digestible in the early stage as compared to 64 percent in the late stage. The decrease in digestibility was greater between the medium and the late stages than between the early and the medium stages.

The dry matter of the grass at the early stage contained 72.10 percent total digestible nutrients while that at the medium stage contained 62.85 percent, and when cut at the late stage 63.62 percent. The nutritive ratios for the three stages were 1:3.39, 1:4.57, and 1:10.10, respectively.

The daily consumption of fresh grass, dry matter, and total digestible nutrients decreased with advanced maturity of the grass. The sheep made larger gains during the digestion trial when fed the immature grass than when fed the more mature forage.

The results of this study indicate that sheep grazing on the range may be expected to consume more total digestible nutrients daily of a higher quality from grass in the early and medium stages than from mature grass, and this should result in a faster rate of increase in growth and weight. A larger area of young grass than of mature grass, however, would be required to produce an equal amount of digestible nutrients.

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DEVELOPMENTAL MORPHOLOGY OF THE GROWING POINT OF THE SHOOT AND THE INFLORESCENCE IN GRASSES [1]

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INTRODUCTION

Many thoroughgoing studies have been made of the development of the grass spikelet, flower, and caryopsis, and of the homologies of the floral parts, but the inception and early development of the inflorescence have received comparatively little detailed investigation. This lack, which exists for other plants as well as for grasses, has been recognized by various botanists (*52, p. 922; 78, pp. 112–113*).[3]

The object of the present investigation was to bring together and summarize as much as possible of the pertinent literature; to trace the morphological changes that take place in a growing point during its transition from the vegetative to the reproductive phase of its development; and to extend and clarify the knowledge of the initiation, organization, and early development of the inflorescence of a number of representative grasses.

This investigation originated about 1920 at the Timothy Breeding Station, North Ridgeville, Ohio, as an outgrowth of an earlier study of the life history of timothy. After 1922 the investigation was continued in the botanical laboratory and greenhouses of Oberlin College, Oberlin, Ohio. Since 1935 some of the observations and illustrations have been made at the Ohio Agricultural Experiment Station, Wooster, Ohio.

REVIEW OF LITERATURE

The previous studies on the early development of the inflorescence of grasses may be conveniently grouped under the following headings: Structural Morphology, Physiological Morphology, and The Plastochrone.

STRUCTURAL MORPHOLOGY

The most extensive of the studies on structural morphology are contained in 6 papers by Trécul (*68, 69, 70, 71, 72, 73*), published in 1880. The first and last papers report comparative studies of the

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[3] Italic numbers in parentheses refer to Literature Cited, p. 515.

developmental succession of branches and spikelets in the inflorescences of 20 or more genera of grasses. The other papers deal with the order of appearance of the primary vessels in the rachis and spikes in 6 genera. Trécul (*68*) described the leaf primordia of the nascent inflorescences of grasses as "bourrelets" or "collars," and the branch primordia, i. e., the secondary protuberances, as "mamelons." He stated that in many species of grasses the primary axis of the inflorescence has its merithalles marked by distichous rudimentary leaves that ordinarily encircle the axis in the form of collars which are higher on the dorsal side, more rarely half-encircling in the upper part of the very young axis. These collars diminish in size as they occur higher and higher on the rachis, and the rachis is often free of them in its upper region. Trécul also state that in *Phleum pratense* L. and in a large number of other grasses the branches develop in succession from above downward on the lower part provided with collars, while they develop acropetally toward the tip.

Goebel (*28*), in 1884, studied the development of the spikelet in 13 genera, and described briefly, and illustrated, early stages in the development of the inflorescence of *Alopecurus ruthenicus* Weinm., *Setaria italica* (L.) Beauv., and *Cenchrus echinatus* L. In his discussion of the latter species, he described the manner in which the protuberances branch and produce shoots of successively higher orders. The following is a translation of his description:

> Each axis of the second order produces two rows of branches. Thus there arises, first of all, a lateral branch to the right and to the left; each of these lateral branches branches again in a plane which crosses the plane of branching of the mother-axis.

Extending the observations of Wigand (*83, p. 90*), published in 1854, Goebel in 1884 (*28*) and 1931 (*30*) has divided the inflorescences of the Gramineae into two types, "dorsiventral" and "radial," the former having their lateral primordia distichous in the same plane as the primordia of the preceding foliage leaves and vegetative buds, the latter having them polystichous. He found the dorsiventral type to be by far the more general. Wigand gave *Zea mays* L. as the only member of the radial type known to him. To this Goebel added *Setaria*, *Cenchrus*, and *Chloris radiata* (L.) Swartz. As examples of "falsely radial" inflorescences, Goebel named *Alopecurus* and *Phleum*, since in these the arrangement of the branch primordia is actually distichous and dorsiventral although the mature inflorescence takes on the appearance of radiality. In the present paper, evidence will be presented indicating that the so-called "radial" type of Wigand and Goebel is also merely "falsely radial."

Trécul described three kinds of developmental succession of lateral primordia in the incipient inflorescence. Goebel, however, saw these as merely three modifications of the developmental relations of acropetally originating organs. These three relations were:

(1) Acropetal primordia and acropetal development, occurring only in the "radial" type of inflorescence: e. g., in *Setaria* and *Zea*.

(2) Acropetal primordia and basipetal development, occurring in *Milium effusum* L., *Poa annua* L., *Glyceria fluitans* (L.) R. Br., *Nardus*, etc.

(3) Acropetal primordia and "a speeding up" of development in the middle region of the inflorescence axis, so that development is basipetal below this region, but acropetal above it, as in *Secale cereale*, *Alopecurus ruthenicus*, and *Phleum pratense*.

Observations on these types of developmental succession are presented in this paper.

Weber (*80, 81*) has recently published two important papers on the origin and early development of the inflorescence of a large number of grasses. He uses the terms acrotony, basitony, and mesotony to designate Trécul's three kinds of development.

Since the work of Trécul and Goebel incidental observations on the origin and early development of the inflorescence of certain of the Gramineae, chiefly cereals, have been made by a number of workers, as adjuncts to other studies. Among these are observations on barley by Lermer and Holzner (*43*) in 1888; on wheat, by Carruthers (*15*) in 1892, Hays and Boss (*33*) in 1899, Jensen (*37*) in 1918, and Kiesselbach and Sprague (*39*) in 1926; on *Hordeum distichum* L. and *Alopecurus geniculatus* L. by Schuster (*66*) in 1910; on wheat, rye, and barley by Schneider (*61*) in 1912; on *Poa pratensis* L., *Phleum pratense*, and *Setaria italica* by Nishimura (*46*) in 1922; on *Oryza sativa* L. by Yamasaki (*87*) in 1928; and on *Monerma repens* Beauv., *Stenotaphrum subulatum* Trin., and *Cornucopiae cucullatum* L. by Helm (*34*) in 1934.

In his description of the nascent inflorescence of wheat, Schneider (*61*), recognizing the relationship of the leaf primordia and the secondary protuberances which they subtend, said: "On the axis of the spike, leaves continue to be initiated. In their axils the primordia of the spikelet axes appear as rounded humps."

Studies have recently been made by Bonnett on barley (*5, 8*) in 1935 and 1938, on wheat (*6*) in 1936, on oat (*7*) in 1937, and on corn (*9*) in 1940, all illustrated with photomicrographs. In his first paper on barley, Bonnett (*5*) correlated the stage of morphological development of the growing point or rudimentary inflorescence with the number of leaves on the shoot; in his paper on wheat he correlated the stage of development with the date on which it was observed.

Noguchi (*47*), in 1929, made an exhaustive statistical study of the developmental changes in the primary shoot in 10 genera of grains, and illustrated and discussed briefly the early stages in the development of the inflorescence and flower. He regarded the apical growing region of the shoot, from the germination of the seed to the expansion of the inflorescence, as the "inflorescence-primordium," treating its development under (*a*) its growth and (*b*) its morphological change.

In the present study, stage *a* is referred to as the "vegetative phase" and stage *b* as the "reproductive phase" or as "the initiation or inception of the inflorescence."

PHYSIOLOGICAL MORPHOLOGY

The effects of various environmental conditions, as, for example, soil nutrients (*22, pp. 14–16*), length of day (*24*), and temperature (*23, fig. 1*), on the vegetative and reproductive development of plants have long occupied the attention of physiologists.

More recently the developmental "aftereffects," induced by temperature, by the photoperiod, and by certain other conditions during germination and early seedling stages, have been extensively studied. Kidd and West (*38*), in 1919, reported on a number of these "induction" conditions and their aftereffects.

In grasses "photoperiodic induction" at germination and its aftereffect during the transition from vegetative to reproductive develop-

ment have been studied especially by Rasumov (*53*) in 1930 and b Lubimenko and Šĉeglova (*44*) in 1931.

Similarly, "thermo-induction," or the aftereffect of germinatio temperature, in relation to the flowering of cereals, has been studied b a number of investigators (*1, 27, 45, 48*). Most of these studie however, have failed to give attention to the fundamental conditior of meristem activity and primordium differentiation.

Purvis (*52*) in 1934 found in winter rye that both short days an low temperature at germination caused an increase in the rate c growth of the apical meristem, and that short days also favore the development of leaves, so that together they brought about a extension of the meristematic cylinder and increase in leaf ridges.

Noguchi (*47*), on the other hand, hinted at a genetic factor when h suggested that the elongated meristematic cylinder is characteristic c perennial forms, while the growing point which continues short an dome- or cone-shaped and in which only a few rudimentary phytomer accumulate, characterizes summer grains. He called the former "th winter grain type," the latter "the summer grain type."

In most of the studies the environmental condition has been re garded as having a specific effect that can be modified only slightly Too little attention has been given to the anatomical condition of th plant preceding reproduction (*55, 85*), to the plant's inherent develop mental sequences and rhythms (*61*), and to the extent to which geneti and hereditary characters determine the plant's responses to a give environmental factor (*54, pp. 675–676*).

In this connection Noguchi (*47*) found that in six species of summe grain the average number of days after seeding, before the morpho logical changes which initiate the inflorescence, ranged from 45 to 85 But in five winter grains he found that the approximate number c days after seeding before morphological changes occurred ranged fror 145 to 175.

THE PLASTOCHRONE

Since the only reference to the plastochrone that has been noted i the American literature is a brief observation by Foster (*26*), in 193 and since the concept seems to offer a valuable approach to the study c certain problems of growth, it has seemed wise to give a brief résum of some of the more important European papers dealing with th subject.

The concept was enunciated and the term first employed b Askenasy (*4*) in 1880.

As a result of observations on the internodes of plants, Sachs ha found that the more constant the environmental conditions the mor uniform was the course of growth. With this as an assumption Sachs (*57*), in 1873, selected a transverse zone 1 mm. long, lying clos to the tip of a root, and measured its growth during successive equa intervals of time through its entire growth period. He found that th increment of growth of this tranverse zone during the equal tim intervals differed in different parts of the growth period.

Askenasy reversed this procedure and studied the time interval required to inaugurate equal, very short, transverse zones or segment of the shoot. He assumed that if growth is approximately uniform under relatively constant environmental conditions the growing point must initiate the extremely short members of the segmented

shoot at constant intervals of time. To this unit time interval he gave the name "plastochrone." He found that the absolute duration of a plastochrone differs in different plants, in different shoots of the same plant, and under different environmental conditions, but that under constant environmental conditions it is "a period of definite and uniform duration."

Almost simultaneously, Westermaier (*82*), in 1881, attacked the same general problem, confining himself to a study of algae, hepatics ferns, and fern allies, all of which have a single apical cell. A translation of his statement (*82, p. 454*) follows:

> As time units I chose the time that elapses from the appearance of one segment wall in the apical cell to the formation of the next succeeding segment wall. I call this time a step. The absolute duration of the time which a step requires is immaterial.

Klein (*40*), in 1884, made an intensive study of the segmentation of the apical cell in 11 species of ferns. He found that the "pace" of apical-cell segmentation is very inconstant.

These approaches to the study of plant growth were almost completely ignored until 1916, when Schüepp (*63*) employed Askenasy's method, extended its application, and confirmed his conclusions.

Schmidt (*60*) in 1924, Schüepp (*64, 65, pp. 792, 793*) in 1926–27 and 1929, Foster (*26, pp. 78, 79, 94*) in 1932, and Priestley and Scott (*51*) in 1933 determined the plastochrone in various dicotyledonous plants.

Almost no studies have been made of the plastochrone in grasses. But Schüepp (*63*), in 1916, recorded the plastochrone of scale or catyphyll initiation in that form of *Bambusa verticillata* which he studied as 2.1 days. The temperature and other conditions of growth were not stated. The plastochrone, at least in some species of grass may be less for those rudimentary phytomers of the inflorescence that are initiated after reproductive processes have begun than for those on the same axis that are initiated while the shoot is in a vegetative condition. Zimmermann (*88*), in 1928, has referred to the plastochrone in *Hypericum uralum*, and Kliem (*41, pp. 285, 286*), in 1937 to the plastochrone in *Avena sativa*.

General observations on the succession of leaf emergence in grasses have been made by different workers. For example (*22, p. 36*), on a number of timothy shoots observed from September 13 to December 9 a new leaf appeared on an average of once in 14.0 days. On another group of shoots observed from April 18 to May 29, when temperature and other conditions were more favorable for growth, the average interval between the appearance of new leaves was reduced to 9.3 days. The difference in the interval at which leaves emerged would suggest the probability of a difference in the plastochrone of phytomer inception and organization under the two conditions.

As the term is used in the present study, the plastochrone is the number of days required for the initiation and organization of a phytomer of a grass shoot up to the time a discernible leaf primordium has developed on it, not to the time of leaf emergence or expansion.

MATERIAL AND METHODS

The appearance of a young grass shoot such as timothy (*Phleum pratense*), when its growing point is at the stage corresponding with the description given below, is illustrated diagrammatically in figure 1

which shows a plant whose growing point is located within the leaves near the base of the shoot and near the surface of the soil.

If the leaves of a young shoot are removed by means of a dissecting needle, the growing point may be found in the form of a minute

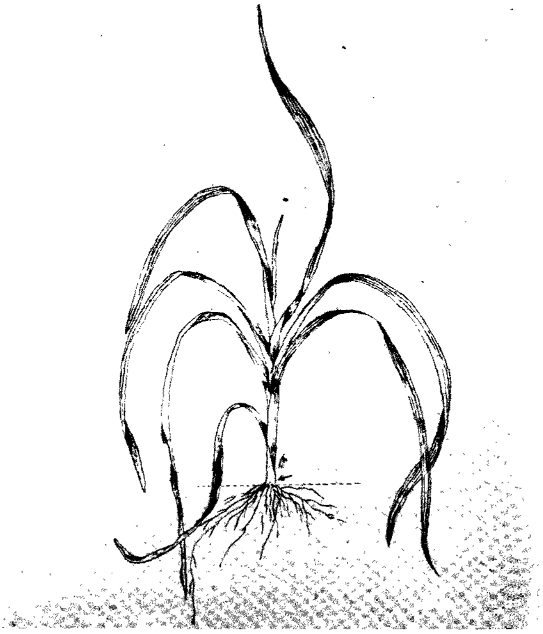


FIGURE 1.—Vegetative shoot of timothy. Dotted line at ground level; arrow directed to the growing point.

slender, often somewhat tapering, meristematic cylinder enclosed by the lower parts of the leaves that have developed.

Most of the illustrations are habit drawings from living material. The method of making them was to dissect away carefully the leaves at the base of the growing points of a number of shoots, to select one or more specimens suitable for illustrative use, and then to examine them under one of the lower powers of a compound microscope. In most instances the drawings were made with the aid of the camera

lucida. If the meristematic cylinder was in a comparatively early stage of development, all parts were drawn under the camera lucida. In older, less translucent and more complex stages, only the outlines were drawn with the aid of the camera lucida; the details were added without its aid. Some of the later drawings were made by the "double-vision" method, and others were entirely freehand.

Since considerable time was required for the study and illustration of a specimen, its tissues were likely to dry out gradually. To prevent this drying, microculture slides with the depression filled with water were used in some cases. The axis of the dissected specimen was severed just below the base of the cylinder by a very oblique cut. The specimen was then placed on the slide in a horizontal position with its base in the water cup. If a small amount of water was kept in the depression, the specimen received enough moisture to keep it in a normal condition, but the cylinder itself was above the water level; thus its appearance was not distorted. In other cases the specimen was supported horizontally on constantly moistened filter paper, the tip projecting beyond this above a film of water on the slide.

During the course of this investigation, a study was made of eight species, in as many genera, belonging to seven tribes (*36*).

GENERAL MORPHOLOGY AND TERMINOLOGY

In order to clarify the terminology employed in this paper, it will be necessary to present some of the conceptions of the morphological units of the vascular plants.

In accordance with the practice of Arber (*3*) and others, the root system and the "stem-leaf" or "shoot" system of Sachs (*58*, *59*) are regarded as the primary units of the plant.

The adult shoot system may be simple or compound. The "simple shoot" stage in the development of a seedling has been called the "primary shoot." But as Sachs (*59, p. 172*) observed, "it often happens that lateral shoots of any order take root and become detached from the primary shoot; they then assume all its peculiarities, and may equally be considered as primary shoots." The compound shoot system consists of the primary shoot plus one or more orders of lateral shoots.

Sachs (*59, p. 595*) extended his conception of the shoot to the inflorescence, where the leaves are generally reduced or otherwise modified as "hypsophyllary leaves or bracts" or "sometimes are entirely absent from the inflorescence or from certain parts of it." Goebel (*29, p. 433*), in 1905, supported this view. On the other hand, Dutailly (*19*), in 1871, insisted that in the Gramineae "the branches of the inflorescence are in no wise comparable to normal shoots." He based his conclusion chiefly on the behavior of the vascular system in the branching of the inflorescence and of the foliage-bearing axes.

In this paper the terminology of Sachs is adopted, and the term "shoot" is used when referring to parts of the inflorescence as well as to the foliage-bearing parts of the plant.

Numerous attempts have been made to subject the "stem-leaf shoot" to analysis, with the object of establishing smaller and more basic units of morphological and physiological organization or of

now existing. For a presentation and review of some of the theories embodying these attempts, the following writers should be consulted: Velenovský (*74, pp. 550–551*), 1907; Cook (*17, pp. 172–176*), 1907; Bower (*10, pp. 134–138*), 1908; Arber (*3*), 1930; and Bower (*11, pp. 544–546, 617–634*), 1935.

For more than a hundred years the segments that compose the shoots of vascular plants have been variously designated as merithalles, phyton units, etc. A considerable number of somewhat different phytonic theories have developed, according to which the shoot is built up of a succession of shoot segments or phyton units, the phyton unit being the true individual. In 1879 Gray (*31, p. 7*) employed the term "phytomer" to designate one of these phyton units or "structures which, produced in a series, make a plant of the higher grade." More recently Weatherwax (*78, p. 40*), 1923, has amplified this definition thus:

"An internode together with the leaf at its upper end, and the bud at its lower end, constitutes a phytomer, the unit of structure of the shoot." The bud may be "small and poorly developed, or sometimes represented by only a meristematic region." He later (*79, p. 212*), 1930, added: "A single leaf arising from the upper end of each internode subtends a bud, or the primordium of one, which is borne in the embryonic region of the lower end of the internode next above it." The leaf and the bud of the same phytomer are therefore on opposite sides of the axis.

For the purposes of this paper the phytomer is accepted as the segmental unit of the plant. In their earliest stages the segmental units of a grass shoot are referred to as rudimentary phytomers.

From the standpoint of taxonomy, Hitchcock (*35, p. 117*) considered the spikelets to be the units of the inflorescences. However, in developmental morphology the phytomers of the inflorescence may be regarded as the units of organization just as the phytomers of which a vegetative shoot is composed may be regarded as the units of its organization.

VEGETATIVE GROWTH

THE GROWING POINT

The terminal growing point of the shoot, or the "vegetative point" of Wolff (*86*), is composed of an apical meristem. The youngest and least differentiated of its cells at the extreme tip of the shoot form the promeristem, from which are directly derived the primary meristems that give rise to the fundamental tissues of the shoot.

According to Douliot (*18, p. 93*), 1891, the promeristem and primary meristems of the growing point of grasses originate in two initial apical cells, the outer initial giving rise to a meristematic dermatogen while from the inner subjacent initial a primary meristem is derived that ultimately differentiates into plerome (central cylinder) and periblem (cortex). On the other hand, Porterfield (*49*), 1930, recognized in the growing point of black bamboo (*Phyllostachys nigra* Munro) four vertically seriated apical initials, the dermatogen and periblem being derived from the two most distal initials, the outer and inner tissues of the plerome from the two lowest.

Through the differential division and growth of the cells of the young plerome, periblem, and dermatogen, a succession of folds, or

ridges, result, as described by Schüepp (*62*) in 1914, Priestley (*50*) in 1928, and Priestley and Scott (*51*) in 1933. These ridges, arising in acropetal succession, constitute the first external indication of a segmental differentiation of the axis into regions composed of node and internode, that is, into the phytomers which constitute the segmented shoot. In grasses the ridges arise on alternate sides of the growing point, forming distichous rows. The apical growing point and elongating meristematic cylinder of the shoot are for convenience regarded as a "vegetative point" until the inception of the first protuberances of the nascent inflorescence. Schmidt (*60*), 1924, Buder (*12*), 1928, and Zimmermann (*88*), 1928, have replaced Hanstein's ambiguous concepts of plerome, periblem, and dermatogen with the concepts tunica and corpus; and Kliem (*41*), 1937, has made rigorous application of these concepts in his detailed study of the vegetative cone of *Avena sativa* and in his critical analysis of Rösler's (*56*) use of these concepts.

FIGURE 2.—Photomicrograph of a growing point of timothy at an advanced stage of the vegetative phase, showing distichous leaf ridges.

When a vegetative growing point is examined with the aid of a good pocket lens, from a direction coinciding with the median plane of the leaves, it appears somewhat translucent with transverse lines or ridges extending across it, giving the cylinder a segmented appearance, the lower ridges being somewhat more conspicuous than those above. When the cylinder is rotated 90° on its axis, it is found that the upper ridges do not completely encircle the cylinder and that they occur in two vertical rows placed 180° apart, the successive ridges alternating with each other. This alternate arrangement of ridges corresponds with the alternate and distichous arrangement of the leaves of the shoot. A photomicrograph of a meristematic cylinder of timothy in its late vegetative phase, viewed at right angles to the plane of the leaves, is shown in figure 2.

The vegetative growing point of grasses ordinarily measures from 0.5 to 1.0 mm. in length. In the species studied the growing points did not usually attain a length of 1.0 mm. until after they had passed the vegetative phase.

As in timothy, so in Kentucky bluegrass (*Poa pratensis*) and many

the time the growing point enters the reproductive phase. The re
is that during its vegetative phase the growing point remains clos
the surface of the ground. On the other hand, in certain grasses, s
as Canada bluegrass (*Poa compressa* L.), even the earliest interno
of the shoot become slightly elongated (*25*, *p. 142*), so that the gr
ing point is soon elevated somewhat above the position indicatec
figure 1.

THE LEAF FUNDAMENT AND EARLY LEAF DEVELOPMENT

Each ridge, the origin of which is outlined above, makes ra
lateral growth until it completely encircles the axis as a somew
oblique leaf primordium or leaf fundament. Meanwhile, the tiss
of the lower or dorsal side of the ridge grow slowly, yet somew
faster than do those of the upper or ventral side, until the ridge hε
narrow free edge.

If conditions are favorable for foliage development the free e
of the ridge finally elongates in the middle region as a leaf-blade fun
ment. For the purposes of this paper, the leaf fundament is a
trarily assumed to have become a young leaf when it exceeds 1 n
in length and has enveloped at least half the axis.

DEVELOPMENT OF INTERNODES AND BUDS

The tissues of the very young phytomers, organizing just be
the apical meristem, remain for a time in a highly meristema
condition with only slight structural differentiation. When furt
structural differentiation sets in, it does not occur uniformly a
simultaneously throughout the phytomer. Instead, the cells of
more distal portion of the axis of each phytomer gradually mat
and become changed into permanent tissues, while most of the c
of the basal region, in proximity to a node, retain their meristema
character for a long time and may continue to add new cells to
axis of the phytomer. The axis is thus composed of intercal
meristems separated from each other by maturing or mature tissu

While the shoot is in the young vegetative condition a bud n
organize from the intercalary meristem at the base of a phytomer,
the side opposite that on which the leaf of the phytomer origina
The bud is, therefore, in the axil of the leaf that crowns the phyto
next below.

LEAF DEVELOPMENT IN RELATION TO INITIATION OF NEW PHYTOMERS

In some species, as teosinte (*Euchlaena mexicana* Schrad.) a
sugarcane (*Saccharum officinarum* L.), the development of the l
primordia into foliage leaves may for a time, under certain conditio
almost keep pace with the formation of new phytomers by the ap
meristem, and the vegetative point may thus retain its hemispher
or rounded conical shape.

In many species new phytomers are laid down by the apical me
stem somewhat faster than leaf development progresses. T
results in a gradual and sometimes rather large accumulation, bel
the undifferentiated vegetative tip, of successive phytomers w
rudimentary leaf fundaments and thus in a progressive elongation of
growing point into a slender meristematic cone or cylinder above
origin of the most distal foliage leaf. The number of rudiment

hytomers that eventually accumulate in the growing point varies idely in different species. For example, in this study there were ound in perennial ryegrass (fig. 5, *B*) accumulations of as many s 17 rudimentary phytomers with more or less well-developed leaf undaments. In quackgrass (fig. 3, *B*) and in timothy (fig. 9, *B*) the umbers may also become quite large. On the other hand, in orchard rass (fig. 14, *B*) only five or six rudimentary phytomers with leaf idges were observed, and the growing points assume the form of a ather slender cone.

THE INFLORESCENCE

An inflorescence in grasses always terminates a vegetative shoot. he rachis is the central axis of the inflorescence (*14, p. 413*). A ranch lateral to this primary shoot is called a secondary shoot of he inflorescence, or a shoot of the second order; one lateral to that s a tertiary shoot, or a shoot of the third order, etc.

The development of the parts of the inflorescence follows fundaentally, though with certain modifications, the same course as the rowth of the vegetative parts of the shoot. Branchings occur, hich may vary in number, in manner of growth, in the extent of longation of their internodes, and in the type of spikelet and floret roduced, the result being mature inflorescences of a great variety f form. Yet, notwithstanding this diversity in the form of the ature inflorescences, their early development is fundamentally the ame.

TIME OF INITIATION OF INFLORESCENCES

The time when the growing point, or meristematic cylinder, underoes the morphological change that definitely marks the initiation of he inflorescence varies with the species and variety, with the order f the shoot and its age, with nutritive and seasonal conditions, with he length of the daily period of illumination, with latitude, and ith other factors.

In the latitude of northern Ohio, the growing point of most perennial rasses, as for example, timothy, ordinarily remains in a vegetative ondition during late summer and fall and until April or very early n May of the following year. About this time there begins to be isible in the growing point certain morphological changes, indicaive of changes in the physiological activities of the cells, that will esult in structures predominantly reproductive in place of those redominantly vegetative.

In annual grasses, whose seeds germinate in the early spring, the nflorescences originate and develop somewhat later than do those of ost perennial grasses.

At the time of "shooting," that is, when the inflorescence emerges om the enclosing leaf sheath, several weeks after the initiation of he inflorescence, the organization and most of the development of he inflorescence have been completed.

TRANSITION FROM VEGETATIVE TO REPRODUCTIVE PHASE OF THE GROWING POINT

In all species that have been studied, the transition from the vegetave to the reproductive phase is usually marked by a sudden and igorous elongation of the growing point and by an increase in its iameter

At about this time, in all parts of the growing point, further development of the leaf fundaments is stopped. However, in the proximal region they may remain as distinct ridges, even in the mature inflorescence. Throughout the middle region, where the ridges are younger and not so well formed, they may gradually become obliterated as the growing point expands in length and diameter, the obliteration beginning on the side of the axis opposite the one on which the midrib of each leaf would have developed (figs. 3, *C*; 9, *C*).

In the growing point of some species, such as perennial ryegrass (fig. 5, *B*) and timothy (fig. 9, *B*), both of which are perennials, there is during the vegetative phase a gradual accumulation of a relatively large number of rudimentary phytomers with leaf ridges, accompanied by a corresponding change from a conical to a cylindrical form. In the growing point of the inflorescences of some other grasses, as in the staminate inflorescence of teosinte (fig. 18, *A*, *B*, and *C*), during the vegetative phase there is an accumulation of only a limited number of phytomers with leaf ridges and the growing point has a conical form up to about the time that transition to the reproductive phase occurs. There is then a rapid elongation of the externally undifferentiated meristem above the leaf ridges, and the growing point rather suddenly assumes a cylindrical form. In a third group of species, the growing points never become entirely cylindrical though they may approach that form. They remain more nearly conical. Orchard grass (fig. 14, *B* and *C*) is included in this group.

BEGINNINGS OF THE INFLORESCENCE

The morphological change in the growing point that marks the lefinite inception of the inflorescence is the formation of lateral swellings or protuberances at the base of some of the rudimentary phytomers. The first protuberances to appear are formed in the axils of the most recently organized and least developed leaf fundaments, and at corresponding positions by the next more distal phytomers that ack leaf fundaments.

Each of these protuberances represents a lateral growing point and nay be regarded as the homologue of the primordium of a vegetative bud (*6, p. 449; 52*). Since it is the primordium of a secondary shoot, or a shoot of the second order of an inflorescence, it may be called a econdary protuberance. In order to emphasize the pronounced morphological differences between the encircling ridge growth of the eaf-base fundaments and the very localized swellings of the primordia of lateral shoots, the latter will be referred to hereafter simply as protuberances. These secondary protuberances are the fundaments f the foundational skeletal structure of the inflorescence.

In timothy and in many other grasses, a large proportion of the phytomers of the nascent inflorescence have more or less well-developed eaf ridges at the time when secondary protuberances begin to form. n such species the number of rudimentary phytomers with leaf undaments varies with the time the shoot has had for vegetative rowth before the initiation of the inflorescence in the spring.

Distal to the region of inception of the earliest protuberances, the pical meristem continues for a time to organize new phytomers cropetally. As each phytomer reaches a certain stage of organiza-
tion it gives rise to a secondary protuberance at its proximal end

without forming a leaf fundament at its distal end; the successive protuberances, therefore, alternate with each other distichously, as is illustrated by the uppermost phytomers in figure 7, *C*. The number of secondary protuberances arising from the hitherto externally undifferentiated apical region of the growing point differs in different species, varieties, and races. This may result in a mature inflorescence most of whose secondary shoots (primary branches) originated in this acropetal succession of protuberances without subtending leaf fundaments. In the inflorescences of other grasses most of the secondary shoots of the inflorescence develop from phytomers with leaf fundaments that accumulated before the inception of the inflorescence.

Simultaneously with this acropetal development, there occurs downward a successive activation of localized meristems, previously dormant, which results in the appearance of protuberances in the axils of successively lower leaf fundaments (figs. 3, *E*; 7, *E*). The strength of this activation lessens basipetally with the more pronounced vegetative organization of the phytomers, the protuberances that arise being successively smaller. In other words, each of the more proximal phytomers in these rudimentary inflorescences is still so physiologically and structurally plastic that the character of its growth activity gradually becomes altered to such a degree that it finally initiates the primordium of a secondary shoot of the inflorescence instead of continuing the growth of the leaf it had already originated. However, the sexualization of the very lowest phytomers of an inflorescence is so greatly delayed and is so incomplete that they are able to form only small, often imperfect or sterile spikelets.

The nascent inflorescence presents the anomaly of having protuberances originating in acropetal succession in the distal region of the meristematic cylinder and at the same time in basipetal succession in the proximal region.

The earliest protuberances at the middle region of the nascent inflorescence begin to develop further before the acropetal and basipetal formation of new protuberances is completed (figs. 3, *E*; 7, *E*).

In most grasses the apical meristem ends its activity by forming a more or less perfect terminal spikelet; but in some cases its activity may cease without the formation of a spikelet (*6, p. 449; 32, p. 177*).

Growth of Secondary Protuberances in Relation to Development of Leaf Primordia

During the vegetative phase of the development of a growing point, that part of each leaf primordium from which the midrib of the blade would develop is usually somewhat higher than the opposite part from which the overlapping free edges of the sheath would develop.

Figure 5, *C* and *D*, shows views of a very young rudimentary inflorescence of perennial ryegrass. Most of the phytomers have formed leaf fundaments; a protuberance has formed in the axil of each of the leaf fundaments except the two lowest. The leaf fundament and the protuberance to which the same number has been given have developed from the same phytomer. The uppermost protuberance has no subtending leaf fundament.

The corresponding stage in orchard grass (fig. 14, *C*) shows more clearly that the strong development of a protuberance at the base of a phytomer is accompanied by the elongation of that side of the phytomer so that the base of the leaf fundament at distal end of the

phytomer is reversed. This is well illustrated in phytomers 2, 3, and 4. If the leaf primordium at the distal end of the phytomer is weakly developed, the vigorous growth of the protuberance may obliterate the overlapping parts of the primordium whereas the midrib region still persists, as is seen in phytomer 5 (*l. pr.* 5 and *sec.* 5). Distal to this, the phytomers have formed only protuberances or the leaf fundaments have been completely obliterated.

Change in Physiological Balance From Vegetative to Reproductive Activity

From the structural relations outlined above, it seems clear that the phytomers in the lower part of any typical nascent inflorescence were being organized while the growing point was in a strongly vegetative state; that during the organization of the middle part, progressive internal changes toward the reproductive state were taking place so that further development of the leaf fundaments of these phytomers was stopped soon after their initiation; and that in the distal part of the rudimentary inflorescence the growing point was in a strongly reproductive state, so that each phytomer organized a protuberance but no leaf fundament (fig. 7, *C, D, E, F*). These figures seem to indicate in the lower part of the primary shoot two physiological and structural gradients running in opposite directions. The first gradient, that of vegetative activity manifesting itself in the origin and development of foliage leaves, passes with lessening strength from the base distally. The second gradient, that of reproductive activity, manifests itself first in the production of protuberances in the middle of the inflorescence, and passes downward for some distance with decreasing strength.

PLANTS WITH ONCE-BRANCHED INFLORESCENCES

Once-branched inflorescences are morphologically the simplest and develop when the secondary protuberances—primordia of the secondary shoots of the inflorescence—do not give rise to lateral protuberances of a higher order, but each instead merely initiates at its distal end a single spikelet. In such an inflorescence all the spikelets are of the second order, terminating shoots of the same order, except the one which may terminate the primary shoot and which is therefore a primary spikelet, or spikelet of the first order.

Typical examples of once-branched inflorescences are those of quackgrass and ryegrass. The pistillate inflorescence of teosinte (see fig. 20) is also once-branched.

The primary spikelets of both once-branched and multiple-branched inflorescences have their glumes and lemmas in the plane of the foliage leaves and of the secondary protuberances of the primary shoot (figs. 3, *F;* 5, *E* and *F;* 7, *F;* 9, *G*). The distichous order of the secondary spikelets is continued distally in the arrangement of the glumes and lemmas of the terminal spikelet, its lowest glume being on the opposite side of the rachis from the uppermost secondary spikelet. In its early organization, the terminal primary spikelet is somewhat precocious. The primordia of its glumes and lemmas are usually laid down somewhat in advance of those on the one, two, or three secondary spikelets immediately below it, and often some time before the secondary protuberances at the base of the inflorescence have begun to be organized.

QUACKGRASS

In most once-branched inflorescences, and also in most or all of the multiple-branched inflorescences, the glumes and the lemmas of the

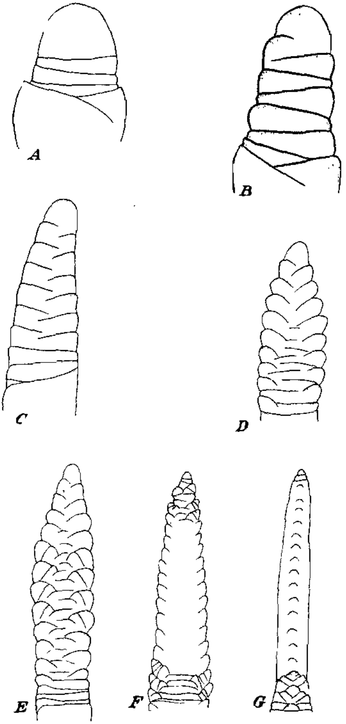


FIGURE 3.—Quackgrass: *A–F*, Seen at right angles to the plane of the leaves; *G*, in the plane of the leaves. *A*, Growing point of a young vegetative shoot, 0.7 mm. long; *B*, more advanced vegetative growing point, 0.9 mm. long; *C*, transition stage, protuberances about to form, 1.0 mm. long; *D*, secondary protuberances forming; *E*, glume primordia on the older protuberances; *F* and *G*, two views, at right angles to each other, of an inflorescence in a late stage of organization, partly in outline, glumes and lemmas forming.

ary spikelets is typified, for the once-branched inflorescences, l quackgrass (*Agropyron repens* (L.) Beauv.). Vegetative growi points and nascent inflorescences of this species are illustrated figure 3, *A–G*.

The earliest indication of spikelet formation occurs when ridg develop on the protuberances, lil those on the older protuberances the middle part of figure 3, *E*. Late additional ridges form (fig. 3, *F* ar *G*). The first ridge is the fundame of the lower glume, the second of tl upper glume. Other ridges becon the lemmas, which develop in acro etal succession above the glume Afterwards protuberances develop i the axils of some or all of the lemma as illustrated by the early protube ances in the axils of the first an second lemmas in the terminal spikel in figure 3, *F*. From these protube ances in the axils of the lemmas, tl florets form.

A fully developed inflorescence quackgrass is shown in figure 4.

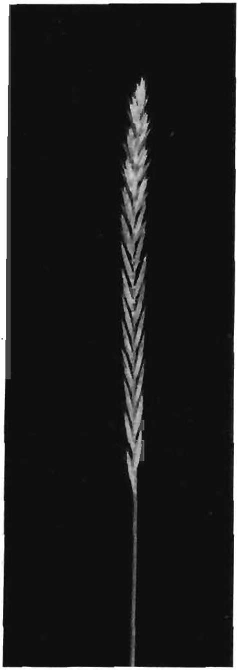


FIGURE 4.—Inflorescence of quackgrass soon after it has emerged from within the enclosing leaf sheaths

PERENNIAL RYEGRASS

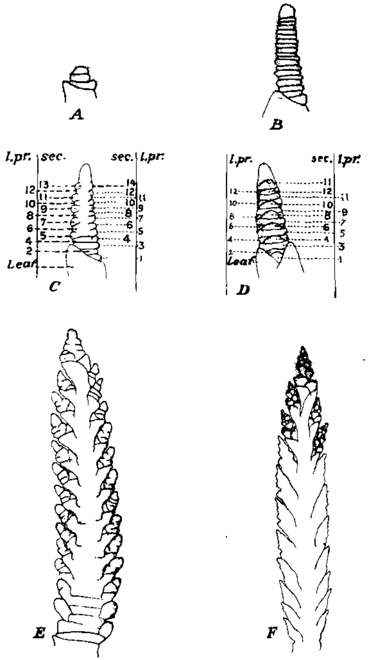
In the once-branched inflorescenc of *Lolium*, unlike those of quackgra and most other grasses, the glumes an lemmas of the secondary spikele originate and develop in the sam plane as the leaves of the primar shoot and as the glumes and lemm of the terminal spikelet. Vegetati growing points and nascent inflore cences of perennial ryegrass (*Loliu perenne* L.) are illustrated in figu 5, *A–F*.

A mature inflorescence of perenni ryegrass is shown in figure 6.

On the lateral spikelets of the rud mentary inflorescences of perenni ryegrass, the primordium of the fir glume, the position of which would b next to the rachis, has been su pressed. However, according to Go bel (*29*, *p. 397*).

In *Lolium temulentum*, especially in the lower flowers of the inflorescence, it frequently developed, seldom as an entire leaf, but usually replaced by two sm leaflets, which are separated from one another by a broad intervening space.

The rudimentary inflorescence shown in figure 5, *E*, had 25 phyt mers producing lateral protuberances or spikelets, as compared wi



URE 5.—Perennial ryegrass: *A*, *B*, *C*, *E*, and *F*, Viewed at right angles to the lane of the leaves; *D*, in the plane of the leaves. *A*, Growing point, early egetative stage; *B*, growing point, late vegetative stage, with 17 rudimentary hytomers below a relatively long, still undifferentiated apical meristem; ' and *D*, two views at right angles to each other of a very young rudimentary nflorescence just after the transition from the vegetative phase. *l. pr.*, leaf rimordia; *sec.*, secondary protuberances. Most of the phytomers have formed af fundaments; protuberances that are fundaments of spikelets have formed ı the axils of all of the leaf fundaments except the two lowest. The leaf fundaıent and the protuberance having the same number have developed from the ıme phytomer; *E*, a middle stage of rudimentary inflorescence; basic skeleton omplete with inception of terminal spikelet; lateral spikelets organizing acropally and basipetally from the middle region; *F*, late stage in organization of ıe inflorescence: florets forming in the axils of the older lemmas.

only 19 in the more advanced specimen illustrated in figure 5, . On any particular date during the period of initiation and formatic of inflorescences, different stages of development may be foun At the same stage of development, there may be a considerable ran in the number of phytomers of which the inflorescences are compose Similar variations have been observed in the other grasses studied.

PLANTS WITH MULTIPLE-BRANCHED INFLORESCENCES

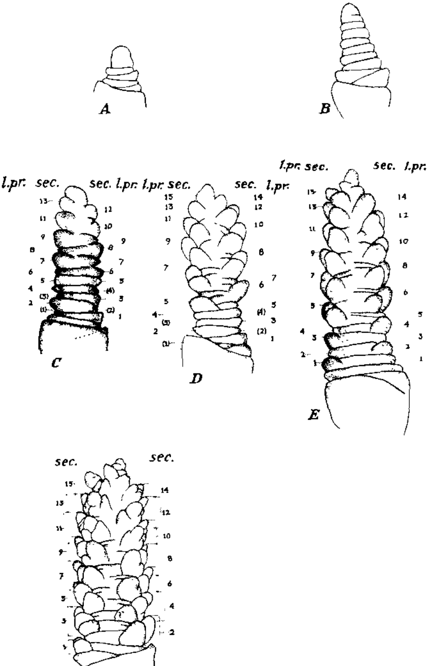
In grasses that are more than once-branched, as canary gras timothy, orchard grass, and others, each secondary protuberance, soo after it is formed, begins to elonga somewhat and to organize tertiar protuberances along its length di tichously and acropetally. These a in a plane at right angles to the plar of the secondary protuberances (fig. *D* and *E*). From the "lateral" face of the tertiary protuberances, as the in turn elongate, quaternary protu berances may develop acropetally, an so on for the higher orders (figs. 9, . and *G*; 14, *F*).

FIGURE 6.—Perennial ryegrass: Mature inflorescence, showing the glumes and lemmas in the same plane as the leaves of the shoot.

Thus a much-brached inflorescenc may result, having each successiv order of shoots at right angles t those of the next lower order. Th number of orders of protuberances and therefore of branches of the in florescence, differs with the species an in a general way is characteristic o the species. As a rule, however, in th species studied the number of order did not exceed four or five.

The extent of internodal elongatio in the inflorescence differs greatly i different species. In some it is s nearly negligible that heads and spike of a compact character result, as in canary grass and in timothy, giving "falsely radial" inflorescences (*28*). In many others the internodal region of some of the phytomers, both of the primary shoot and of its branches, elongate to such an extent as to form axes of considerable length. This may result in loose and often relatively large inflorescences, as, for example, the inflorescences of tall oatgrass and orchard grass, and the staminate inflorescence of teosinte.

Organization of the latest and youngest protuberances of a given order does not necessarily precede the initiation of protuberances of the next higher order. Instead, before the youngest secondary protuberances have been initiated, tertiary protuberances begin to form on the older secondaries and even quaternary protuberances may have



IGURE 7.—Canary grass: All figures at right angles to the plane of the leaves; leaf primordia (*l. pr.*) and secondary protuberances (*sec.*) that are a part of the same phytomer have the same number assigned to them. *A*, Growing point of a young vegetative shoot; *B*, growing point at an advanced vegetative stage; *C*, a very early stage in the initiation of an inflorescence; the three proximal phytomers of the inflorescence proper, like the uppermost culm phytomers, are well advanced in vegetative organization; *D*, tertiary protuberances forming on the older secondaries; *E*, the terminal spikelet organizing its two glumes; the beginnings of quaternaries at phytomers 11 and 12; *F*, primordia of lemmas on terminal spikelet and of glumes on older lateral spikelets. For convenience in the correlation of *C*, *D*, *E*, and *F*, the structures that are a part of or arise from corresponding phytomers are given the same numbers. If at a certain stage a particular phytomer has not yet initiated the secondary protuberance which the corresponding phytomer of another older shoot shows, as in *E*, *secs.* 1, 2, 3, and 4, then the potential but not yet developed secondary of the younger stage is given the corresponding number enclosed in parentheses, as in *C* and *D*, *secs.* (1), (2), (3), and (4).

begun to form on the older tertiary protuberances. Thus, pro berances and ultimately spikelets of all orders may be forming sim taneously in different parts of the inflorescence.

Canary Grass

Different stages in the development of growing points of can grass (*Phalaris canariensis* L.) are illustrated in figure 7, *A–F*.

In figure 7, *F*, the lateral groups arising from phytomers 10 and appear to be the most advanced. Acropetally from these the gro from phytomers 12, 13, 14, and 15 are less and less advanced, althou most of these seem to be nearer the stage of spikelet organization tl are any of those below phytomer The rudimentary terminal prim spikelet is more advanced than any the lateral ones developing from s ondary protuberances. Below phy mer 10, the development of the lat groups is basipetally less and less vanced, decreasing as the size of subtending leaf base increases.

Figure 8.—Inflorescence of canary grass

A fully developed inflorescence shown in figure 8.

Timothy

Vegetative growing points and n cent inflorescences of timothy (*Phle pratense*) are illustrated in figure *A–G*.

Photomicrographs of four represe ative stages in the development the timothy shoot and inflorescer are shown in figure 10, *A–D*.

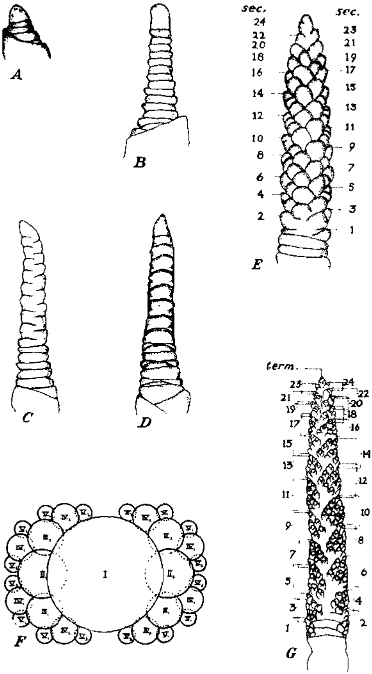
A fully developed inflorescence timothy is illustrated in figure 11,

In a small percentage of inflor cences of timothy, the leaf fundament crowning the uppermost int node of the culm, such as the one next above the basal leaf fundam in figure 9, *C–D*, may have a prolonged development and produc short leaf sheath, with a more or less well-formed blade, subtendi the group of spikelets of the most proximal phytomer of the matı inflorescence. Figure 11, *B*, illustrates this.

Tall Oatgrass

The development of the vegetative growing point and of the you inflorescence of tall oatgrass (*Arrhenatherum elatius* (L.) Mert. a Koch) is shown in figure 12, *A–F*.

The vegetative growing points of the primary shoots of tall oatgr gradually become cylindrical in form, not greatly unlike those quackgrass and perennial ryegrass. The final number of phytom of which it is composed does not become so great as in the two otl species mentioned, both of which are once-branched. Instead, in t



IURE 9.—Timothy: *A*, *B*, *C*, *E*, and *G*. viewed at right angles to the plane of he leaves; *D*, in the plane of the leaves. *A*, A young, cone-shaped vegetative growing point; *B*, a cylindrical growing point at a late stage of the vegetative phase; *C* and *D*, two views of a nascent inflorescence just after the transition rom the vegetative phase; the right of *C* is the face of *D*; the relatively large, vell-established leaf fundament at the base crowns the second internode of the ulm below the inflorescence; the next higher leaf fundament crowns the uppernost internode of the culm; *E*, a middle stage in the organization of the infloescence; formation of inflorescence phytomers and of secondary protuberances early complete; tertiaries forming but not quaternaries; *sec.*, secondary prouberances; *F*, diagrammatic vertical projection of cross sections of two successive phytomers of a young inflorescence of timothy, showing the arrangement of protuberances of different orders. The rachis is represented by I; two secondary axes by II_1 and II_2; and two axes of each higher order which have originated rom each secondary one by III_1, III_2, IV_1, IV_2, V_1, and V_2. As these subsequent orders of protuberances develop, they become crowded from the positions which hey otherwise might occupy; *G*, late stage in the organization of the infloresence; inception of terminal spikelet (*term.*) and of lateral spikelets of different rders; all structures within a single bracket originated from one phytomer

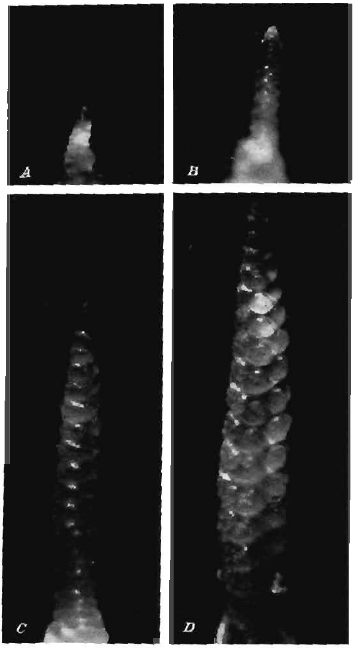


FIGURE 10.—Timothy: Photomicrographs of vegetative growing points and young inflorescences, all viewed at right angles to the plane of the leaves and of the secondary protuberances. *A*, Young vegetative growing point at a slightly more advanced stage than the one illustrated in figure 9, *A*; *B*, growing point at about the time of transition from vegetative to reproductive phase—about the stage illustrated in figure 9, *C*; *C*, a nascent inflorescence with secondary protuberances throughout the middle region; *D*, a young inflorescence in which the two rows of prominent protuberances in the foreground are tertiaries, developed on the flanks of the two rows of secondaries seen less clearly in the background on the extreme left and right.

oatgrass provi multiplicati spikelets is through the d ment of bran higher orders than through tinued increase number of sec spikelets.

Tall oatgrass trates a pheno common an grasses with lo florescences, in the proximal mer or phytom the second branches do n ways elongate; of the tertiary even quater branches may re grouped at the of the seconda forming clusters pseudowhorls branches at the n of the rachis. more distal intern of the branches, ever, lengthen co erably, giving ris an inflorescence of paniculate ty (fig. 13).

ORCHARD GRASS

In the infloreseen of many species grasses, the branc or the spikelets both, as in orch grass, grow toward o side of the axis which they are bor On the branches of t staminate inflore cence of teosinte, als the spikelets gro toward one side.

Different stages in the development of the vegetative growing poi and of the nascent inflorescence of orchard grass (*Dactylis glomerat* L.) are illustrated in figure 14, *A–H*.

A fully developed inflorescence is illustrated in figure 15.

The vegetative growing point of the grasses studied usually, if not always, soon becomes slightly compressed, but without any apparent distinction between the two faces. In orchard grass, even when the earliest secondary protuberances are forming (fig. 14, *C*), there are still few if any characters distinguishing the two sides. When, however, the tertiary protuberances begin to form (fig. 14, *D* and *E*) the primary shoot is already strongly bifacial. This becomes even more pronounced at later stages and extends to the shoots of the higher orders, finally constituting one of the characteristic features of the mature inflorescence. Thus the one-sided character of the spikelet clusters is initiated at about the time when the secondary protuberances of the rudimentary inflorescences begin to produce branch protuberances of higher orders.

FOXTAIL MILLET

In the inflorescences of the foregoing species of grasses, each protuberance ultimately organizes a spikelet at its distal end. In foxtail millet, however, some of the protuberances of the higher orders elongate into sterile bristles (*28, p. 19*).

Various stages of the vegetative and reproductive phases of foxtail millet (*Setaria italica* (L.) Beauv.) are illustrated in figure 16, *A–M*.

The specimens illustrated in figure 16, *A–K*, are from shoots dissected in late September and early October. In the nascent inflorescence of figure 16, *B*, the irregularities in the primordia appear to be due to the initiation of protuberances. In the nascent inflorescence shown in figure 16, *C*, distal to the three phytomers crowned with leaf fundaments, there are very rudimentary reproductive phytomers without leaf fundaments. Two of these phytomers are initiating groups of three protuberances on their visible side. The central and uppermost protuberance of each group is situated in the same plane as the leaf apex of the basal phytomer.

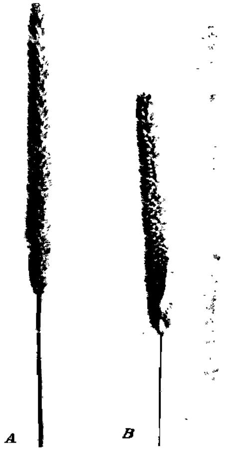


FIGURE 11.—Timothy: *A*, A typical inflorescence; *B*, an inflorescence in which the most proximal group of spikelets is subtended by a reduced leaf.

The positional relations of the members of each group are such as to suggest that the central one of the three is an incipient secondary shoot and that the other two are the first and second tertiaries at the base of its lateral flanks. This simultaneous initiation of the secondary shoot and its two lowest tertiaries, when taking place on the alternate sides of acropetally successive phytomers, would result in the appearance of protuberances in six longitudinal rows (fig. 16, *D*, *E*, and *F*), a condition characteristic of millet (*75*).

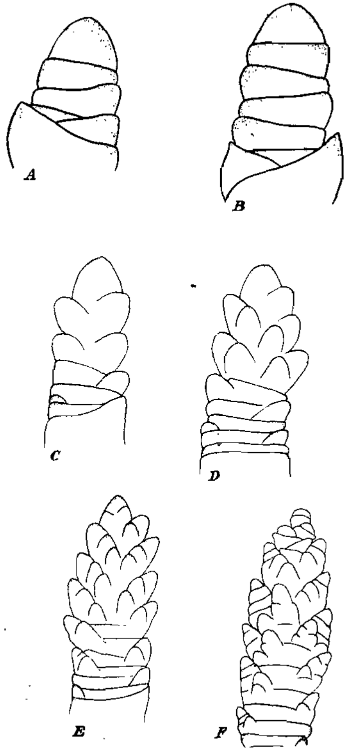


Figure 12.—Tall oatgrass. *A* and *B*, Vegetative growing points at different sta of development; *C*, nascent inflorescence; secondary protuberances are form *D*, the earliest tertiary protuberances are being organized on the lateral face the older secondaries, while additional secondary protuberances are contint to develop acropetally and basipetally; *E*, additional tertiary protubera have formed, and the glumes on the terminal spikelet have begun to devel *F*, quaternary protuberances are developing on the flanks of some of the ol tertiary branches. The terminal spikelet is more advanced and specialized t any of the lateral ones, protuberances representing florets having appeared in axils of its two oldest lemmas.

With approaching cessation of apical elongation of the primary axis, the extremely short secondaries and tertiaries rapidly initiate groups of protuberances of higher orders (fig. 16, *G*), which later develop into groups of spikelets and bristles, disposed in six longitudinal rows (fig. 16, *H*), and in the mature inflorescence shown in figure 17.

In order to obtain more precise evidence concerning the stages shown in figure 16, *B* and *C*, studies were made in a later year of the growing points of vigorous plants that had been seeded in the open in early June. Strong shoots dissected in late July had formed a considerable number of rudimentary phytomers above the highest foliage leaf; secondary protuberances were being initiated in advance of their tertiaries. At the young stage shown in figure 16, *L*, the secondary protuberances were being initiated acropetally and basipetally from the middle region and the older ones were showing a decided extension of one flank. At the slightly older stage presented in figure 16, *M*, the primodria of two tertiary protuberances were visible on the flanks of each of the older secondaries.

FIGURE 13.—Inflorescence of tall oatgrass.

Goebel (*28*) and others have classified the inflorescences of millet and certain other grasses as belonging to the "radial" type. These inflorescences were supposed to be polystichous instead of distichous as in most grasses. The observations on millet made in this study show that the apparently polystichous groups actually originate from secondary protuberances having a distichous and alternate arrangement, as such groups do in timothy. These results indicate that millet should go in Goebel's "falsely radial" type, along with *Alopecurus*, *Phleum*, etc., the inflorescences of which are actually distichous.

TEOSINTE

In some grasses, as teosinte, the staminate and pistillate florets are in separate inflorescences, which have different forms. The pistillate inflorescence of teosinte (*Euchlaena mexicana*) has already been referred to under the description of once-branched inflorescences. The staminate inflorescence is multiple-branched with elongated internodes; different stages in its develop-

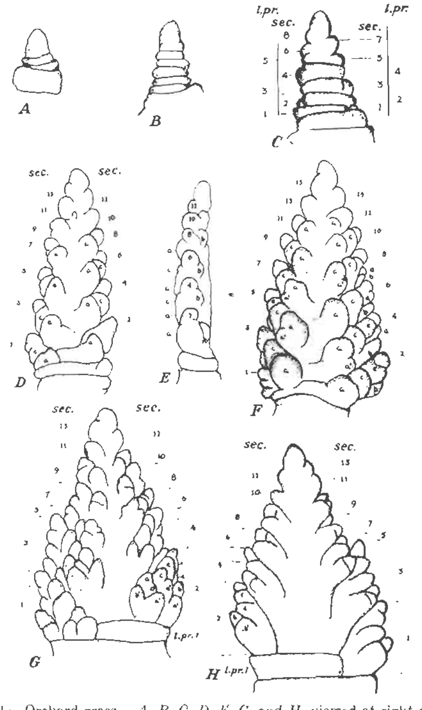


FIGURE 14.—Orchard grass. *A*, *B*, *C*, *D*, *F*, *G*, and *H*, viewed at right angles the plane of the leaves and secondary protuberances; *E* in the plane of t secondaries. Leaf primordia (*l. pr.*) and secondary protuberances (*sec.*) numbered from the base upward; *a*, *b*, *c*, etc., are successive tertiaries; *a'*, *c'*, successive quaternaries; *A* and *B*, two vegetative growing points at differe stages; *C*, a nascent inflorescence, soon after transition from vegetative pha *D* and *E*, a face and an edge of a rudimentary inflorescence at the stage in wh. tertiary protuberances are forming on the secondaries; *F*, a slightly older florescence, the face of which corresponds with that of *D* and *G*; quaterna protuberances are just being initiated on some of the tertiaries; *G* and opposite faces of a still older rudimentary inflorescence, sometime before t branches had begun to elongate; the right side of *G* is the left of *H*; the organi tion of the foundational skeleton of the inflorescence is now nearly comple each numbered bracket includes a secondary with the tertiaries and quat naries arising from it.

The first indication of the transition from the vegetative to the reproductive phase and of the inception of the inflorescence is an especially vigorous growth of the apical meristem of the growing point, *resulting in a relatively long, externally undifferentiated* meristematic cylinder, slightly compressed in cross section and with a broadly rounded summit. Following or coincident with this first spurt of elongation, secondary *protuberances* begin to form at the base of the cylinder (fig. 18, *B* and *C*).

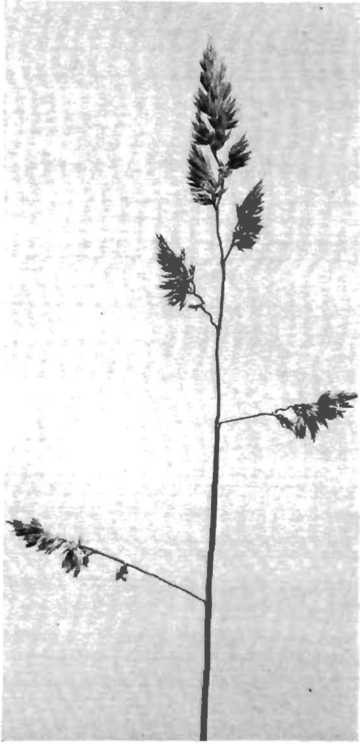


FIGURE 15.—Fully developed inflorescence of orchard grass.

The *process* of initiation and development on the central axis of secondary protuberances and their tertiaries (*42*) continues in acropetal succession as shown in a much later stage in figure 18, *D* and *E*. This figure also illustrates the pronounced bifaciality of the primary shoot, which, together with the frequent tendency to droop in its nature condition, has caused it to be regarded as a secondary branch (*77, p. 320*). In this inflorescence the two proximal secondaries and their lowest tertiaries have developed into elongated lateral shoots, of which only the stumps are shown. Above these the secondaries and tertiaries have not elongated as branches. Instead, each secondary organized directly into a pediceled spikelet and is tertiary into a sessile spikelet. Similarly, the proximal elongated secondary shoots (fig. 18, *F–J*) organize their tertiaries into pediceled spikelets,

shoots organize their quaternaries into pediceled spikelets, their quinaries into sessile ones.

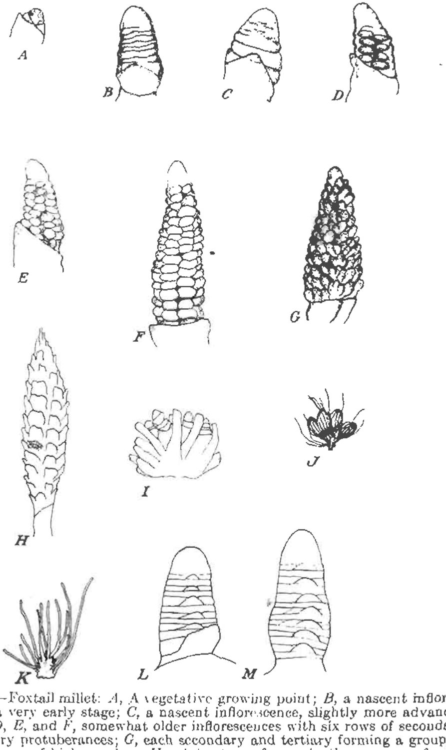


FIGURE 16.—Foxtail millet: *A*, A vegetative growing point; *B*, a nascent inflorescence at a very early stage; *C*, a nascent inflorescence, slightly more advanced than *B*; *D*, *E*, and *F*, somewhat older inflorescences with six rows of secondary and tertiary protuberances; *G*, each secondary and tertiary forming a group of protuberances of higher orders; *H*, a late stage of organization; groups of rudimentary spikelets and bristles in longitudinal rows; diagrammatic; *I*, a group of rudimentary spikelets and bristles, adaxial view; *J*, a group of mature spikelets and bristles, abaxial view; *K*, a group of bristles without spikelets, from the base of the inflorescence; *L* and *M*, nascent inflorescences of vigorous shoots; *L*, with secondary protuberances; *M*, tertiaries forming.

During the development of the rudimentary staminate inflorescence the tertiary shoots, originating from tissue at the extreme base of a secondary shoot, may be separated from the secondary by the expanding tissue of the primary axis. Very slight displacements of this kind occur in the inflorescence shown in figure 19, at secondaries 2, 4, and 5, and are shown diagrammatically in figure 18, *K*. In the panicles of some other grasses the most proximal tertiary shoots may also be displaced upward on the rachis for considerable distances above the secondary shoots from which they originated, in the manner described by Strasburger (*67, p. 118*).

A pistillate inflorescence with staminate spikelets in the distal part is illustrated in figure 20. The spikelets, each consisting of one floret with its silk (style and stigma), are arranged alternately in two ranks. This distichous arrangement is continued into the distal part of the inflorescence, where each phytomer gives rise to two staminate spikelets instead of a single pistillate spikelet.

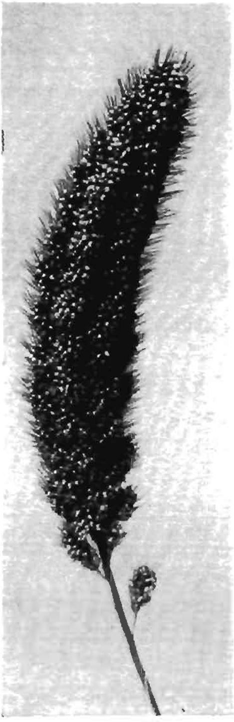


FIGURE 17.—Mature inflorescence of foxtail millet.

LATER DEVELOPMENTS IN THE INFLORESCENCE IN RELATION TO PHYTOMER INITIATION

One might expect that spikelet and floret formation would progress in the same order as phytomer organization. Actually this is not the case. It has already been pointed out that in general the earliest secondary protuberances appear in the middle region of the incipient inflorescence and that new protuberances are successively formed distally and proximally to these. However, further development of spikelets and florets takes place more rapidly in the distal regions than in the middle and basal regions of the young inflorescence. The exact order and extent of this progress differs in detail with different species.

Corresponding with the more rapid specialization and sexualization of the most distal and youngest phytomers and with the early development of spikelets and florets in that region, flowering and maturing of seed also begin there (*21, figs. 1, 2; 32, p. 15; 76, p. 1062;*

84, p. 24). The way in which blooming begins near the apex and p
gresses more or less regularly toward the base of an inflorescence of t
European species *Phalaris truncata* Guss. is illustrated in figure 21

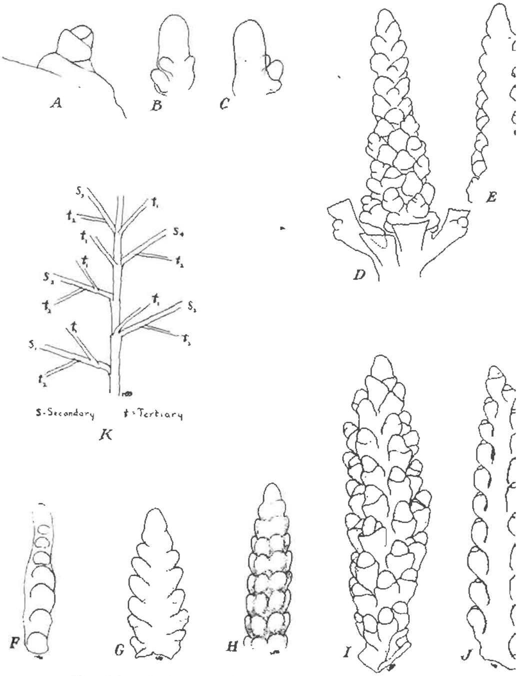


FIGURE 18.—Teosinte: *A*, A vegetative growing point; *B* and *C*, opposite fac
of an inflorescence at its inception; *D* and *E*, opposite faces of the inflorescen
at a considerably later stage of development; *F* and *G*, two views, edge and fac
of a very young secondary shoot with tertiary protuberances, from the low
part of an inflorescence; *H*, an older secondary shoot with both tertiary a
quarternary protuberances, abaxial view; *I*, an abaxial view of a seconda
shoot at stage of spikelet organization; *J*, an adaxial view of another secondai
shoot at about the same stage as *I*; *K*, skeletal diagram of the lower part of t
inflorescence shown in figure 19.

That the last florets to bloom or the last seeds to mature in a

plained for many inflorescences by the fact that they originated om the phytomers which were the last to produce secondary pro-iberances. That this is not the complete explanation, however, is dicated by the fact that the most distal secondary protuberances iginate later than those near the middle of the inflorescence, yet ie florets near the apex may all be past bloom while in the middle gion large numbers are still blooming (fig. 21).

In some grasses, e. g., timothy, the latter phenomenon may be plained in part by the fact that near the apex of the inflorescence the

FIGURE 19.—Staminate inflorescence of teosinte.

condary protuberances produce, through branching, relatively few otuberances of higher orders, while throughout the middle part of ie inflorescence the protuberances continue for some time to sub-vide and produce higher orders of branches and spikelets.

On the other hand, the relatively early flowering and early maturing seed near the tip of the inflorescence may be due to the gradual ropetal diminution in the number and size of the vascular bundles

by Bugnon (*13*), in 1920, and Arber (*2*), in 1928. Or, more specifically, to a condition of the phloem in the bundles, resulting in a "natural girdling" as was suggested by Roberts and Wilton (*55*), in 1936, for dicotyledons.

DEVELOPMENT OF EITHER VEGETATIVE OR REPRODUCTIVE PARTS FROM THE SAME RUDIMENTARY PHYTOMER

To determine whether an individual rudimentary phytomer of a grass shoot remains for some time capable of either vegetative or reproductive development, depending upon the conditions for growth, timothy was grown under short and under long days.

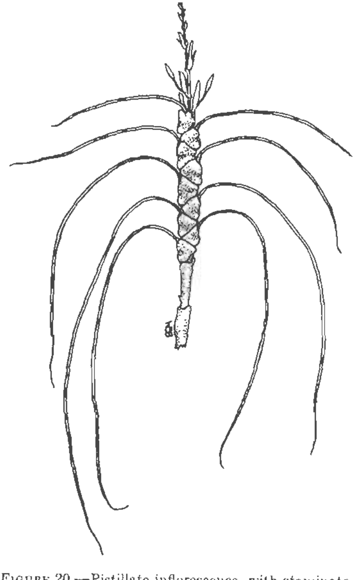


FIGURE 20.—Pistillate inflorescence, with staminate spikelets in distal parts of teosinte.

A number of plants were transferred from the field to the greenhouse on December 1, 1925. They grew under natural length of day until January 9, 1926, then with 7 hours' illumination each day until January 27, 1926. Each plant was then divided into two parts, one of which was grown under short-day, the other under long-day conditions, until March 10, 1926. The short-day plants were covered with a dark box from 4 p. m. until 9 a. m., and were thus illuminated for 7 hours each day. The long-day plants were illuminated artificially, from about sunset until midnight each day, by means of an ordinary 200-watt electric-light bulb suspended about 30 inches above the surface of the soil in which the plants were growing; they were thus illuminated for a total period of about 17 hours each day. All the plants continued to grow with natural illumination from March 10 to April 14.

For observation, shoots were selected that had started their development at approximately the same time, had produced the same number of leaves per shoot, and were therefore relatively uniform.

When the plants were first placed under artificially controlled lengths of day, the leaves were removed from several typical shoots and a count was made of the number of rudimentary phytomers in the growing point of each; there was an average of 14.75 rudimentary phytomers per growing plant.

Final records were obtained from four shoots grown under short days and from four shoots grown under long days.

Under short days, all shoots remained in a vegetative condition and the number of leaves continued to increase until the close of the experiment; an average of 8.5 new leaves per shoot developed from January 27 to April 14. Under long days during the same period, an average of 1.5 new leaves formed and on each shoot an inflorescence developed.

By subtracting the number of leaves that developed on the long-day plants from the number that developed on the short-day plants, it is evident that on an average seven phytomers in corresponding positions produced leaves under short days but under long days became a part of the inflorescence.

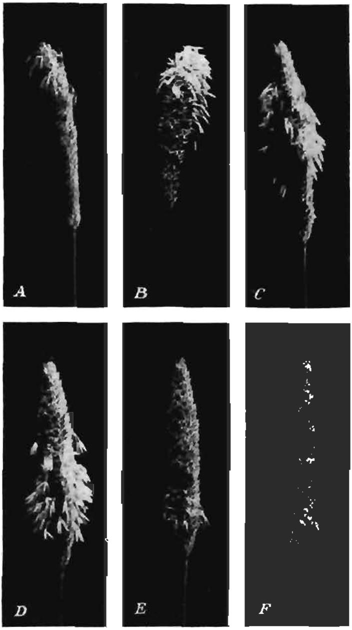


FIGURE 21.—Inflorescence of *Phalaris truncata* Guss. on different days during the blooming period: *A*, First day; *B*, second day; *C*, seventh day; *D*, ninth day; *E*, eleventh day; *F*, fifteenth day.

The results demonstrate quite conclusively that, for some time after the formation of a rudimentary phytomer in the growing point of a grass shoot, it remains capable of developing either vegetative or reproductive parts according to the conditions for growth.

THEORY IN REGARD TO DISTICHOUS ORIGIN OF PARTS OF INFLORESCENCE IN ALL GRASSES

In the representatives of the seven tribes of the grass family that have been studied during this investigation, it has been found that

assumes in its fully developed state, its initiation is always marked by the formation of protuberances arranged distichously in the same plane as the leaves of the shoot. This fact seems to justify the conclusion that in all grasses the distichous arrangement of the leaves and of the primary branches on the vegetative part of the shoot is continued into the inflorescence without change in the sequence of homologous parts.

It is generally thought that in maize (*Zea mays* L.) the pistillate inflorescence, or ear, and also the central axis of the staminate inflorescence, or tassel, have a polystichous phyllotaxy (*28; 81, p. 90*). Three or four theories have been advanced by different botanical writers and investigators as explanations of the way in which this polystichous arrangement originated (*16; 78, pp. 110–113*). However, the investigations described herein indicate that it is probable that in maize, as in other grasses, the arrangement of the spikelets on the central axis is fundamentally distichous.

SUMMARY

A study was made of the vegetative growing point of eight species of grasses, belonging to seven tribes; of the morphological changes that take place in the growing point during its transition from the vegetative to the reproductive phase; and of the organization and early development of the inflorescence.

In the vegetative part of a grass shoot each internode, together with the leaf at its upper end and the bud or potential bud at its base, constitutes a phytomer. The young phytomers of the vegetative growing point are called rudimentary phytomers. Their leaf fundaments are at first slight transverse ridges, which quickly encircle the rudimentary axis.

The growing point is at first cone- or dome-shaped, slightly compressed, usually a fraction of a millimeter long, consisting of a very few (usually one to three) rudimentary phytomers below the undifferentiated apical meristem.

The apical meristem lays down the rudimentary phytomers, one after the other, in acropetal succession and at rather regular intervals of time; this constant period is known as the plastochrone.

There may be such a lag in leaf formation that the number of accumulated rudimentary phytomers gradually increases and the growing point assumes a cylindrical form. This occurs in such species as quackgrass, perennial ryegrass, timothy, and tall oatgrass.

In certain other species, e. g., in the staminate inflorescence of teosinte, only a slight accumulation of rudimentary phytomers occurs during the vegetative phase, but at the transition to the reproductive phase the apical meristem elongates very rapidly into an externally undifferentiated cylinder from which the greater part of the inflorescence develops.

One of the earliest indications of the inception of an inflorescence is the appearance of swellings or protuberances. In many grasses they arise at about the middle region of the growing point, usually with a distichous arrangement, and continue to form for some time in both acropetal and basipetal succession. In the basal or proximal part of the inflorescence, each protuberance is in the axil of a ridge or leaf primordium; in the apical or distal part, they form without subtending ridges. These secondary protuberances have the same relative posi-

tion on the primary axis as the vegetative buds and are their homologues.

In some grasses, such as quackgrass, perennial ryegrass, and others, the secondary protuberances do not give rise to protuberances of higher orders. In many other grasses, such as timothy and orchard grass, the secondary protuberances do give rise to protuberances of successively higher orders.

In some grasses, such as foxtail millet, the primary branches, that is, the secondary shoots, of the inflorescence seem to arise polystichously. However, evidence obtained in this investigation indicates that fundamentally the secondary shoots of inflorescences of this type originate distichously, as in other grasses.

Environmental conditions largely determine whether vegetative or reproductive development takes place. For many grasses, length of day is one of the most important of these conditions. In timothy, under short days, all of the rudimentary phytomers developed vegetatively; under long days, from rudimentary phytomers in corresponding positions on the growing points, reproductive development occurred.

As a rule, each protuberance, whatever its order, ultimately organizes a spikelet at its apex. There is a general tendency for spikelet formation to begin in the upper middle region of the young inflorescence and to progress both acropetally and basipetally. Further specialization, however, is more rapid in the distal region than in the middle and proximal regions. Consequently, flowering and seed maturation progress from the apex toward the base of the inflorescence, in the reverse order to phytomer formation.

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A COMPARATIVE STUDY OF THE SEASONAL ROOT DEVELOPMENT OF SOME INBRED LINES AND HYBRIDS OF MAIZE [1]

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INTRODUCTION

In an endeavor to gain further knowledge of the root system of corn (*Zea mays* L.), an investigation was planned to include both the developmental and genetic aspects. Some of the primary objectives were as follows: (1) To obtain a detailed record of the growth of the root system of corn from planting to maturity; (2) to determine the range in root characters among certain inbred lines; (3) to ascertain how these root characters are expressed in hybrid combinations; and (4) to identify any characters possibly associated with the force required to pull a corn plant from the ground.

HISTORICAL REVIEW

Pavlychenko (*16*)[3] recently published an excellent review of the various methods employed in the past in the study of root systems. In addition to his own "soil-block" washing method, he described 11 different procedures used by previous investigators, viz, direct washing, trench washing, steel-frame washing, soil-prism washing, concrete-compartment washing, nail-and-needle-brush washing, Hellriegel's steel cylinder, water-culture and soil containers, observation pit, direct tracing, and Weaver's trench method. Sayre [4] used the "lithium" method to determine the extent of corn root systems. After lithium had been placed in the soil at definite distances from the plant, the stalk was tested for its presence. By this means he hoped to determine the lateral extension of the root system. The results, however, failed to agree with observations made by the direct tracing of the roots. Sayre suggested that his results may more nearly represent the absorptive area of the corn root system than the maximum extension of some of the individual roots.

The majority of the investigations relating to the roots of corn have been primarily concerned with the distribution of the roots in the soil. The reports vary widely as to the extension of the root system vertically and horizontally. Hickman (*7*), in Pennsylvania, and Farris (*3*), in New Jersey, found that the root system was relatively shallow. The maximum distance of penetration in New Jersey was only 2 feet,

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[3] Italic numbers in parentheses refer to Literature Cited, p. 537.

[4] SAYRE, J. D. THE LITHIUM METHOD OF MEASURING THE EXTENT OF CORN ROOT SYSTEMS. 1937 [Unpublished.]

both laterally and vertically. Other reports on the distribution of the roots of corn have come from Morrow and Hunt (*15*), in Illinois, Hays (*6*), in Minnesota, King (*11*), in Wisconsin, Ten Eyck (*24, pp. 333–346; 25*), in North Dakota and Kansas, Shepperd (*20, pp. 529–535*), in North Dakota, Miller (*14*), in Kansas, and Weaver et al. (*26, 27*), in Nebraska. This group of investigators found corn roots at depths ranging from 4 to 8 feet and with lateral extensions ranging from 2 to 4 feet. In all cases the corn root system extended to considerably greater distances than those reported from Pennsylvania and New Jersey. Several of these workers noted that the crown roots from the lower nodes extended almost horizontally from the culm, whereas the roots from the higher nodes often extended nearly vertically into the soil.

Less attention has been devoted to the seasonal development of the root system of corn than to the distribution in the soil of the roots of a mature plant. Hickman (*7*) obtained measurements of the size of the root systems at 2, 4, 6, and 8 weeks after planting. In general, his observations during this period of growth agree with those in the present study. Weihing (*28*) examined corn roots 2, 4, 6, 8, and 12 weeks after planting, both in pots and in the field. Rotmistrov (*18*) found that from the "bushing" to the flowering period maize roots averaged a daily growth of 1.5 cm. vertically and 0.8 cm. horizontally. Bensin (*1*), in Czechoslovakia, observed that the maximum extension of the roots was reached at the time of tasseling.

In connection with the lodging of corn, the study of the root system has naturally assumed some importance. Holbert and Koehler (*8*) designed a plant-pulling machine to determine the force required to pull a plant from the ground. Koehler, Dungan, and Holbert (*12*) observed that an inbred strain of corn with the ability to stand erect possessed a root system about twice as large as that of a strain inclined to lodge. Hayes and McClelland (*5*) found that—

> * * * where parents (inbred lines) were widely different in ability to withstand lodging, the F_1 was, in general, of intermediate habit, although there were some exceptions to this rule. When both parents had low lodging indices, the F_1 crosses were also low and when both parents had high lodging indices, the F_1 crosses lodged severely, as a rule.

Wilson (*30*) noted that the presence of many brace roots and short distances between the lower nodes of the corn plant were important in the prevention of lodging. Pettinger (*17*) found that potassium and phosphorus were especially beneficial in promoting strong root growth. The weather, however, was found to be a more important factor in the determination of lodging than either the fertilizers or the cropping systems employed. Hall (*4, p. 26*) observed that lodging was determined by a complex of morphological factors and—

> * * * that strong lines were strong because they possessed one or more characters that correlate highly with erect plants and in spite of the fact that they also possessed certain characters that might be associated with weak lines.

Literature relating to purely genetical studies of root characters is scarce. The only gene definitely identified in connection with a root character of corn has been described by Jenkins (*9*). Plants exhibiting the effects of this gene have fewer primary (or main) roots than has ordinary corn. Kiesselbach and Weihing (*10*), studying mature plants, noted the comparative root development of selfed lines of corn

and their F_1 and F_2 hybrids. Hybridization markedly stimulated the penetration, spread, length, and diameter of the roots in the first generation, whereas those of the second generation were intermediate in these respects. Smith (*23*) noted that a high ratio of secondary roots to primary roots was the rule in "phosphate efficient" lines and that in the F_1 a high ratio of secondary to primary roots was dominant over a low ratio.

Some attention has been given to the seminal root system of corn. Wiggans (*29*) reported that dent corn and popcorn usually have four "temporary" roots (including the radicle). Flint and sweet corns usually have only one temporary root, the radicle. Siemens' (*21*) results were similar to those of Wiggans, except that he found Michigan popcorn among those types that have no secondary seminal roots. Smith and Walworth (*22*) believed they had accumulated rather strong evidence for a significant positive correlation between yield and seminal root development, but Collins (*2*) advanced certain criticisms against their methods. Mangelsdorf and Goodsell (*13*) failed to find a correlation between seminal root development and yield in four out of five tests.

MATERIALS AND METHODS

The strains selected for the root studies consisted of four inbred lines, 56, 4–8, 51, and 84; two single-cross hybrids, 56 × 4–8 and 51 × 84; and one double-cross hybrid, (56 × 4–8) × (51 × 84). The four inbred lines are at present being widely used in Ohio in the commercial production of hybrid seed corn. Line 56 matures at about the average date at Wooster, Ohio, line 4–8 later than average, and lines 51 and 84 approximately 10 days earlier than average.

The planting was made in the spring of 1937 at the Ohio Agricultural Experiment Station at Wooster as part of the regular farm rotation of corn–oats–wheat–alfalfa. The soil type, Wooster silt loam, is derived from sandstone and shale, glaciated, and naturally well drained.

The average monthly precipitation and temperature during the 1937 season at Wooster closely approximated the 40-year average.

The planting was arranged in four replications of three-row plots. The individual plants were separated by a minimum distance of 40 inches.

The planting was made on May 27, and the first root samples were obtained 16 days later. The samples consisted of five root systems of each strain, and they were taken at random on the same day of each succeeding week until 15 weeks after planting. The root systems were removed from the ground in a cylinder of soil 16 inches in diameter and 10 inches in depth, securely wrapped with burlap, and thoroughly soaked prior to removal of the soil with a fine spray of water. Practically no rootlets were lost by this washing process. Obviously, a cylinder of soil of these dimensions did not include the entire root system, but direct tracings of the roots by the trench method indicated that the great bulk of the root system, including practically all of the fibrous portions, was obtained.

Direct tracing of the crown roots, by the trench method, was accomplished for one plant of each strain at three times during the season.

THE SEMINAL ROOT SYSTEM

DRY WEIGHT, COMBINED LENGTH, AND NUMBER OF SEMINAL ROOTS

Table 1 gives the mean values obtained for the measurements of the seminal roots of the inbred lines and hybrids from the second to the seventh week after planting. The dry weights of the roots of the inbred lines reached a maximum at about the fourth week and those of the hybrids at about the fifth week, after which time their dry weights decreased. At 3 weeks, the first internode of many of the plants began to show severe rot injury. With the partial or complete death of the tissues in this region, it is doubtful whether the seminal root system was any longer active in absorption. At least 5 weeks before the crown root systems reached their maximum growth, the seminal root systems were entirely dead. It is believed that this premature death of the seminal roots was due entirely to the many injurious insects and fungi present in the field soil.

TABLE 1.—*Average weight, length, and number of seminal roots per plant in the inbred lines and hybrids from the second to the seventh week after planting*

Inbred line or hybrid	Average values for seminal roots per plant from the second to the seventh week after planting		
	Dry weight	Total length	Roots [1]
Inbred lines:	*Gram*	*Centimeters*	*Number*
56	0.062	53.3	3.9
4-8	.080	46.4	4.0
51	.085	45.5	3.4
84	.053	45.9	3.8
Minimum significant difference [2]	.020	12.4	.37
Hybrids:			
56 × 4-8	.143	73.7	4.7
51 × 84	.133	64.0	4.3
(56 × 4-8) × (51 × 84)	.155	67.9	4.3
Minimum significant difference [2]	.038	10.9	.41

[1] Including the radicle.
[2] Minimum difference for significance calculated as twice the standard error of a difference.

Even 16 days after planting, the seminal roots of the hybrids exhibited marked hybrid vigor, in contrast to the roots of the inbred lines. This was apparent in the fact that the hybrids had a greater number of roots, with a greater dry weight and combined length. The hybrids showed significant differences only in respect to number of roots, whereas the inbred lines showed significant differences in number and dry weight (table 1). Consequently, the differences between the seminal roots of the inbred lines were reflected between the hybrids only in respect to the number of roots.

It was of interest to note in what degree the seminal root development of the various strains correlated with the development of the crown roots and the shoot. Within neither group of strains was it possible to predict the relative number of crown roots from the number of seminal roots, nor was it possible to predict the relative dry weight of the crown roots from the dry weight of the seminal roots. Likewise, the information on the number or the dry weight of the

eminal roots did not permit a reliable estimate of the potentialities or development of the top of the plant or the yield of grain.

DISTRIBUTION OF SEMINAL ROOTS IN THE SOIL

The direct tracing study by the trench method at 30 days after ılanting indicated a predominantly horizontal distribution of both he radicle and the secondary seminal roots. Maximum distances f penetration observed for the radicle were 4 inches vertically and inches horizontally. The secondary seminal roots occupied a posiion similar to that of the radicle.

THE CROWN ROOT SYSTEM

COMPARATIVE DEVELOPMENT OF CROWN AND SEMINAL ROOTS

The crown roots began a rapid growth soon after germination and n a short time far surpassed the seminal roots in dry weight. At ; weeks after planting, the crown and seminal roots were about equal n dry weight. At only 6 weeks after planting, the crown roots were ıt least 25 times as heavy as the seminal roots, and for one strain he ratio was as great as 55 to 1.

DRY WEIGHT OF CROWN ROOTS DURING THE GROWING SEASON

The increase in the dry weight of the crown roots of both the nbred lines and the hybrids is illustrated graphically in figure 1, *A*. Γhe data form a typical S-shaped growth curve. Distinct differences in dry weight could be observed at 16 days after planting, and ıt 15 weeks most of the strains were widely separated. When fully leveloped the largest root system (56 × 4–8) was twice the dry weight of the root system of the largest inbred line (4–8) and four times that of the root system of the smallest inbred line (51). The ratio of the lry weights of the roots between the two groups of strains, however, remained at a value of about 2 or 3 during most of the season.

The maximum dry weight of the crown roots was attained at ıpproximately the time of silking for the majority of the strains. Γhere was some indication that the maximum dry weight was attained he week following silking for two of the strains. Larger samples of each strain would be required to ascertain this point with greater ıccuracy.

The maximum dry weight of roots attained by a particular inbred ine was related in some degree to its time of maturity. For example, ine 4–8 was the latest of the lines to mature and had the greatest dry weight of roots. Line 51 was the earliest and had the least dry weight of roots. Line 84, however, was nearly as early as line 51 but ıad a considerably greater dry weight of roots. It would, therefore, seem that at least among the inbred lines certain hereditary factors other than time of maturity influenced the maximum dry weight of the crown roots. Among the hybrids the maximum dry weight of roots increased in the order of the lateness of maturity of the strains.

Differences in dry weight of roots among the inbred lines were reflected in the differences among the hybrids. The double-cross hybrid had a root weight somewhat intermediate between the single-cross hybrids, but, as in many other root characters, it most nearly resembled the single cross 56 × 4–8.

A differential seasonal response seems to occur among the four inbred lines, as indicated by the dry weights of roots at 10 weeks and 13 weeks after planting (fig. 1, *B*). On this basis lines 84 and 4-8 should resist lodging best, followed by lines 56 and 51. At 13 weeks

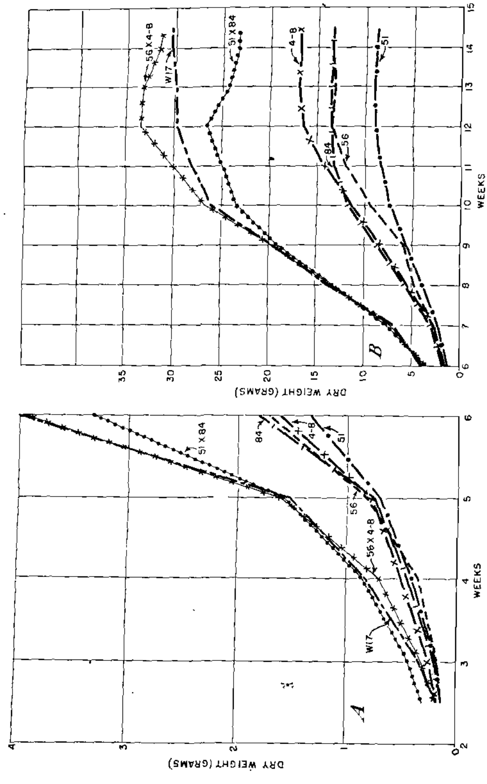

FIGURE 1.—Dry weight of the crown roots of inbred lines and hybrids from (*A*) the second to the sixth week and (*B*) from the sixth to the fifteenth week after planting. W17 is the double-cross hybrid (56 × 4-8) × (51 × 84.) Curves smoothed by computing a moving average of 3 weeks' data.

after planting the expected order would be: 4-8, 56, 84, and 51. No data are available to test the differential seasonal root development

of the growth curves of the dry weights of roots of the hybrids shows that they differed little in dry weight up to 9 weeks after planting but that the differences increased after that time. Consequently, the possibility of a differential seasonal response with respect to lodging is likewise suggested for the hybrids.

TOTAL LENGTH OF MAIN ROOTS OF CROWN ROOT SYSTEMS DURING THE GROWING SEASON

The seven strains of corn differed in several root characters in addition to dry weight. Table 2 gives the mean total length of the main roots of the crown root systems for two different periods during the growing season. Obviously, the hybrids had a considerably greater total length of main roots than did the inbred lines. Within each group of strains, the differences are not so pronounced as they were in regard to dry weight. Line 51, for example, is the only one of the inbred lines differing significantly from the other lines in the total length of the main roots of the mature root systems. The total length of the main roots of the hybrids reflects a situation closely resembling that for dry weight of roots. The single crosses differed significantly in total length of the main roots of the mature root systems, but the double-cross hybrid differed significantly only from cross 51 × 84.

TABLE 2.—*Mean total length in centimeters per plant of the main roots of the crown root systems for the inbred lines and hybrids during two different periods of the growing season*

Inbred line or hybrid	Mean total length per plant of main roots of crown root systems for period indicated	
	Second to ninth week, inclusive	Eleventh to fifteenth week, inclusive [1]
Inbred lines:	*Centimeters*	*Centimeters*
56	395.0	931.5
4-8	384.7	894.7
51	292.8	528.9
84	379.6	932.5
Minimum significant difference [2]	59.96	69.19
Hybrids:		
56 × 4-8	528.4	1,239.0
51 × 84	479.9	1,051.3
(56 × 4-8) × (51 × 84)	470.3	1,200.5
Minimum significant difference [2]	55.60	146.85

[1] Beginning with the tenth week, length measurements were not taken on the roots of the lower three nodes because of the injury which they suffered from insects and root rots.
[2] Minimum difference for significance calculated as twice the standard error of a difference.

NUMBER OF "FUNCTIONAL" MAIN ROOTS DURING THE GROWING SEASON

Roots which entered the soil were arbitrarily termed "functional." The root systems of the hybrids exceeded those of the inbred lines by a considerable margin both in dry weight and total length of the main roots; but this was not true with respect to the number of functional main roots (fig. 2). For this latter character, the root system of one inbred line (4-8), when fully developed, exceeded that of any of the hybrids. The root systems of the hybrids, however,

showed only small differences in this character, but again 56 × 4-8 and the double-cross hybrid were more nearly alike than were 51 × 84 and the double cross. Each single cross more nearly resembled the inbred parent having the larger number of main roots than it did the inbred parent having the smaller number of main roots.

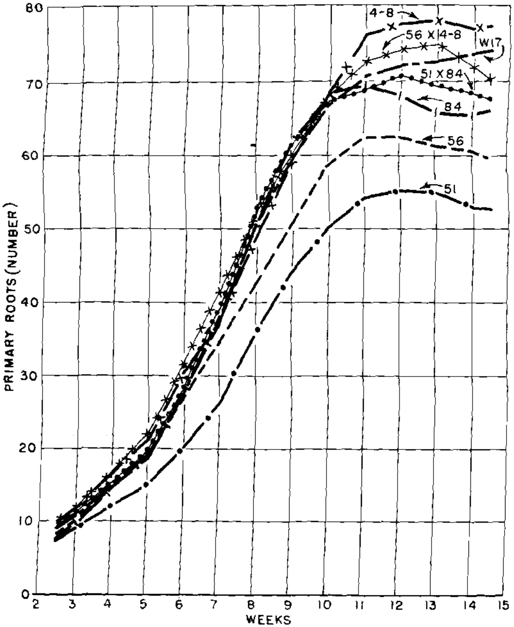

FIGURE 2.—Number of main roots in the crown root systems of inbred lines and hybrids of maize from the second to the fifteenth week after planting. W17 is the double-cross hybrid (56 × 4-8) × (51 × 84). Curves smoothed by computing a moving average of 3 weeks' data.

Differences in the number of functional main roots were evident at 16 days after planting and remained fairly consistent during the growing season. The mean number of main roots among all the strains ranged from 5.5 to 7.7 at 16 days and from 50.0 to 79.2 at 15 weeks. This root character, like dry weight and total length of main roots, reached a maximum value at or slightly subsequent to the time of silking.

ROOT DEVELOPMENT AT DIFFERENT NODES

CHARACTERISTICS OF CROWN ROOTS AT EACH NODE

The crown root system of corn develops by successively higher ıorls of roots at closely set nodes. Theoretically, the scutellar node the lowest node of the plant, but for practical purposes, the lowest de that gave rise to a whorl of crown roots was designated as the st node in this study. Successively higher nodes with whorls of ɔwn roots were numbered in the order of their occurrence. Despite e proximity of these lower nodes, they are nearly always distinishable, and rarely is any difficulty encountered in determining their act sequence. The number of main roots per node increases rather ›wly upward to the third or fourth node and then more rapidly at e nodes higher on the culm. A typical sequence of number of main ɔts for a mature plant of 56 × 4–8 from the first to the tenth node ıs as follows: 4, 4, 4, 4, 5, 7, 9, 12, 16, 13.

GURE 3.—Root systems of inbred lines and hybrids of maize 2 weeks after planting: *a*, Line 56; *b*, line 4–8; *c*, line 51; *d*, line 84; *e*, single-cross hybrid 56 × 4–8; *f*, single-cross hybrid 51 × 84; *g*, double-cross hybrid (56 × 4–8) × (51 × 84).

The main roots arising from the first three or four nodes were of a latively small and uniform diameter throughout their length (fig. 3). ıese roots early develop an extensive growth of fine lateral rootlets. ; the main roots from each higher node appear, there is a progressive crease in diameter, but this thickness is not maintained throughout eir length. The earliest and most vigorous growth of lateral root-s occurs proximally unless the main root sustains severe injury ɔm some source. In that event, dependent upon the strain of corn ncerned, the most vigorous growth of lateral rootlets may occur just oximal to the point of injury.

Probably one of the most significant facts revealed in this root ıdy is that strains of corn differ tremendously both in the ordinary oduction of lateral rootlets and in their production subsequent to e severance of the main root by some insect pests. Two of the in-

bred lines employed in this study, 4–8 and 51, had a small number of lateral rootlets and the other two lines, 56 and 84, were plentifully supplied with them. These differences are readily seen in figure 4. Such variations in root growth must mean very great differences in

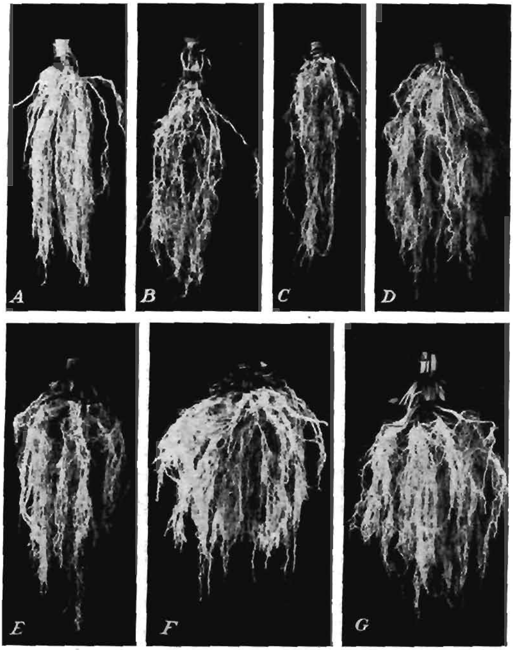

FIGURE 4.—Root systems of inbred lines and hybrids of maize 11 weeks after planting: *a*, Line 56; *b*, line 4–8; *c*, line 51; *d*, line 84; *e*, single-cross hybrid 56 × 4–8; *f*, single-cross hybrid 51 × 84; *g*, double-cross hybrid (56 × 4–8) × (51 × 84).

the total area of root surface in contact with soil particles, and, consequently, may be an important factor in soil, air, water, and nutrient relationships with the plant. The fact that the inbred lines show a differential response in lateral root growth following insect injury may be of importance in areas where corn is damaged as a result of the activities of such insect pests as the southern corn rootworm.

NUMBER OF WHORLS OF "FUNCTIONAL" CROWN ROOTS DURING THE GROWING SEASON

At 16 days after planting the number of whorls of crown roots (fig. 5) ranged from 1.7 to 2.0, and at 15 weeks after planting they

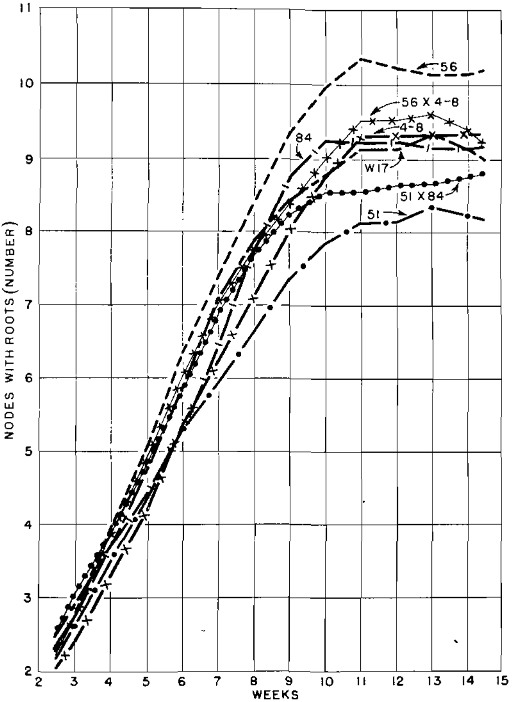

FIGURE 5.—Number of nodes per plant with main roots from the second to the fifteenth week after planting. W17 is the double-cross hybrid (56 × 4–8) × (51 × 84). Curves smoothed by computing a moving average of 3 weeks' data.

ranged from 8.0 to 10.4. In general, during the 1937 season about one additional whorl of roots developed each week for the first 8 or 9 weeks after planting.

In total number of nodes with roots, one of the inbred lines (56 again exceeded any of the hybrids. The fully developed root syste of this line had slightly more than 10 nodes with roots, whereas th most poorly developed inbred line (51) had only 8 nodes. The singl cross hybrids exhibited a significant difference of nearly 1 whole noc with roots in their fully developed root systems. One single-cro hybrid (56 × 4–8) most closely resembled the inbred line 4–8 with th smaller number of nodes with roots, whereas the other single-cros hybrid (51 × 84) seemed to be about intermediate to its componer inbred lines in this root character. The single-cross 56 × 4–8 and th double-cross hybrid failed to differ significantly in the maximu number of nodes with roots.

TOP-TO-ROOT RATIOS DURING THE GROWING SEASON

At 16 days after planting, the top was one to two times heavie than the root system (fig. 6). Throughout the season this dispropo tion between the dry weights became constantly greater until 1 weeks after planting, when the top-to-root ratio was more than 20: for some of the strains. The ratios for the single-cross hybrids wer approximately intermediate between the ratios of their inbred parent up to the time of silking, when the hybrid ratios tended to equal c exceed those of the inbred parent with the larger ratio. The doubl cross hybrid exhibited a higher ratio than either of the single-cros hybrids up to 9 weeks after planting. Its ratio then became inte mediate between the two single-cross ratios and finally appeared t be nearly identical with that of cross 56 × 4–8.

Several investigators have reported on the top-to-root ratio of cor at various periods during the growing season. Hickman (*7*), i Pennsylvania, found the ratio to be 0.6:1 for plants 2 weeks old an 3.2:1 for plants 4 weeks old. The corresponding average values fc the hybrids in this study were 1.4:1 and 2.7:1. King (*11*, *Rpt. 9* in Wisconsin, reported the ratio at maturity to be 6.7:1. Mill (*14*), in Kansas, found the ratio at maturity to average 9.6:1 i 1914 and 7.8:1 in 1915. Schweitzer (*19*), in Missouri, reported th ratio to be 23.7:1 on September 10. Weihing (*28*), in Nebrask found the ratio to be 6.4:1 for a small variety grown to maturity i pots. Rotmistrov (*18*, *p. 51*) reduced the relationship to a gener law by stating that—

> * * * at the beginning of the development the weight of the roots exceed the weight of the superficial parts, in the middle part of the development, th relation approximates 1:1, and at the end of the development the weight of th superficial parts exceeds that of the roots.

This law did not seem applicable to the strains of corn included i this study, since even at 2 weeks after planting the top in all case outweighed the roots.

In general, the top-to-root ratios of the strains in this study ex ceeded the ratios reported in previous studies. At 15 weeks afte planting, the smallest ratio was 12:1 and the largest 23:1.

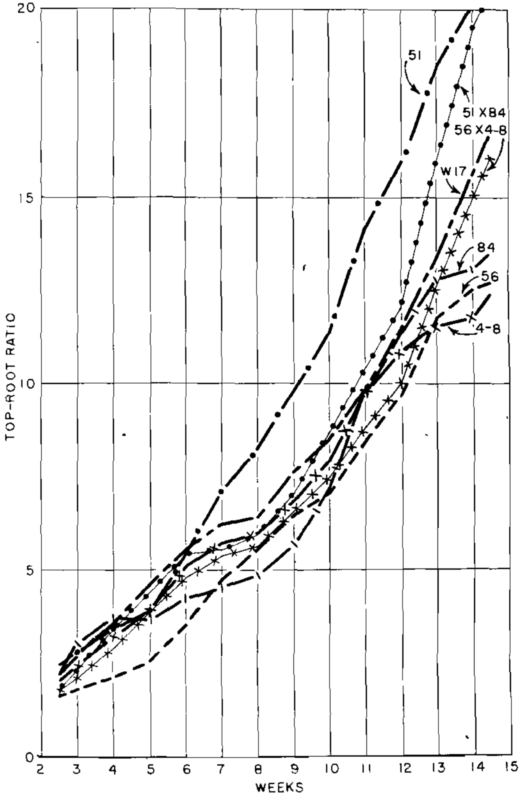

FIGURE 6.—Top-to-root ratios, based on the dry weights, of inbred lines and hybrids of maize from the second to the fifteenth week after planting. W17 is the double-cross hybrid (56 × 4–8) × (51 × 84). Curves smoothed by computing a moving average of 3 weeks' data.

DISTRIBUTION OF MAIN ROOTS OF CROWN ROOT SYSTEMS IN THE SOIL DURING THE GROWING SEASON

The main roots of the crown root systems were traced for one plant of each strain at 30, 49, and 87 days after planting. The trench method was employed in making the excavations.

At 30 days after planting, the main roots of the inbred lines had not quite penetrated halfway through the 7 to 12 inches of surface soil. Both the crown roots and the seminal roots extended nearly horizontally from the culm. The maximum lateral spread observed was 12 inches. No marked difference was evident between the inbred lines and the hybrids at this period either in the depth of penetration or in the lateral spread.

At 49 days after planting, most of the roots of the inbred lines had penetrated to a maximum depth of 10 to 12 inches and extended laterally to slightly greater distances. Line 51 had a root system that was markedly restricted in distribution. The roots of the hybrids seemed to be somewhat more extensively distributed at this period than the roots of the inbred lines. Maximum distances of penetration were 2 feet both horizontally and vertically.

The last examination of the root systems was made 87 days after planting. There seemed to be no significant changes in maximum values for the depth and lateral extension of the main roots since the 49-day examination.

It is apparent that the root system of corn extended to relatively short distances at Wooster during the 1937 season. The results agree with those obtained by Hickman (*7*), in Pennsylvania, and Farris (*3*), in New Jersey. The studies of King (*11*), Shepperd (*20*), Miller (*14*), and Weaver (*26*), in Wisconsin, North Dakota, Kansas, and Nebraska, respectively, indicate, however, that under certain soil and climatic conditions corn may be much more deeply rooted.

PULLING RESISTANCE AND SOME ASSOCIATED ROOT CHARACTERS

A plant-pulling machine was used to determine the force required to pull a single root system from the ground. This machine was equipped with a single movable pulley operating between the scales and a stalk attachment device.[5] Twenty plants of each strain were pulled on August 14, 11 weeks after planting.

All the hybrids surpassed the inbred lines in mean pulling resistance (table 3). The pulling resistance of the lines ranged from 158 pounds to 227 pounds and that of the hybrids from 290 pounds to 350 pounds. The low value for line 51 was expected on the basis of its poorly developed root system. The double-cross hybrid was nearly identical with the single-cross hybrid 56 × 4–8 in pulling resistance.

Mean values for some of the characters that might have been influential in promoting strong rooting are given in table 3. Correlation coefficients were calculated within strains between (1) pulling resistance and dry weight of the crown root system and (2) pulling resistance and the number of main roots (table 4). Significant positive correlations within five strains were found between pulling resistance and root weight. No significant correlations were evident between pulling resistance and number of main roots.

[5] *Designed by A. A. Bryan, Division of Cereal Crops and Diseases.*

ABLE 3.—*Mean pulling resistance and mean values for some associated root characters in the inbred lines and hybrids of maize*

Inbred line or hybrid	Mean values for—								
	Pulling resistance	Dry weight of crown roots [1]	Crown roots [1]	Nodes with roots [1]	Plant height [1 2]	Vertical distance on culm with functional roots [1]	Above-ground nodes with functional roots [1]	Diameter of roots [1 3]	Diameter of culm [1 4]
	Pounds	*Grams*	*Number*	*Number*	*Centimeter*	*Centimeter*	*Number*	*Millimeters*	*Centimeters*
ıbred lines:									
56	227	13.1	60.1	10.1	174	7.6	1.2	2.22	2.16
4-8	209	17.4	78.0	9.3	200	7.6	1.2	2.45	2.62
51	158	8.9	53.6	8.1	197	4.7	.7	2.10	2.22
84	188	13.4	66.3	9.2	141	4.8	1.1	2.02	2.12
Minimum significant difference [5]	39.0	1.3	4.4	.3	6.0	.9	.3	.13	.15
ıybrids:									
56 × 4-8	350	32.0	72.1	9.4	264	8.9	1.7	2.92	3.06
51 × 84	290	23.3	67.5	8.6	245	5.3	1.0	2.77	2.74
(56 × 4-8) × (51 × 84)	333	30.0	72.8	9.1	263	7.1	1.3	2.83	2.94
Minimum significant difference [5]	51.0	3.3	4.8	.4	5.0	1.4	.3	.12	.13

[1] Mean values obtained from samples taken from weeks 12 to 15.
[2] Measured to the tip of the tallest leaf when stretched vertically.
[3] *The mean root diameter was obtained by measuring the diameter of one main root at each node at a* listance of 15 cm. from the culm.
[4] The diameter of the culm was measured at the middle of the internode immediately beneath the highest ıode bearing *functional roots and was determined as the mean of the smallest and largest diameters.*
[5] Minimum difference for significance calculated as twice the standard error of a difference.

TABLE 4.—*Correlation coefficient within strains between pulling resistance and the dry weight of roots and between pulling resistance and the total number of main roots*

Inbred line or hybrid	Correlation coefficient between—		Inbred line or hybrid	Correlation coefficient between—	
	Pulling resistance and dry weights of roots	Pulling resistance and number of roots		Pulling resistance and dry weights of roots	Pulling resistance and number of roots
56	[1] 0.50	0.15	56 × 4-8	0.38	0.18
4-8	[1] .53	-.45	51 × 84	.31	.21
51	[2] .75	-.12	(56 × 4-8) × (51 × 84)	[2] .68	.43
84	[2] .72	.53			

[1] Significant.
[2] Highly significant.

Dry weight of roots was closely associated with pulling resistance (table 3). The only other root character that appeared to be related to pulling resistance among both inbred lines and hybrids was root diameter. Among the inbred lines as a group and the hybrids as a group, the vertical distance on the culm with "functional" roots seemed to bear a relation to pulling resistance. A similar situation existed in regard to the number of nodes with roots. Pulling resistance seemed to be more closely related to the number of above-ground nodes with functional roots among the hybrids than among the inbred lines. Plant height and culm diameter seemed to bear little rela-

tion to pulling resistance among the lines, but among the hybrids the tallest plants with the thickest culms had the greatest pulling resistance.

There was hardly sufficient lodging in the plots during the 1937 season to establish any marked differences among the strains. In lines 56, 4–8, 51, and 84, respectively, 5.5, 2.6, 37.1, and 2.2 percent of the plants lodged at an angle of 45° or greater in November. In hybrids 56×4–8 and 51×84 and the double-cross hybrid, respectively, 0.3, 2.1, and 1.9 percent of the plants lodged. Line 51 failed to remain erect even in a season unfavorable to lodging. This might have been predicted on the basis of its extremely poor root development.

SUMMARY

A comparative study was made of the seasonal development of the corn root system by the use of a double-cross hybrid, two single-cross hybrids, and the four inbred lines employed in making up the hybrids.

The seminal root systems of the hybrids were larger than those of the inbred lines, but in both groups of strains they died early in the growing season, probably because of injuries sustained from various insect larvae and root diseases. There was no consistent correlation, within either group of strains, between the development of the seminal roots and the development of the crown roots or the tops.

Marked differences were noted among the strains in regard to number, dry weight, and total length of the main roots of the crown root systems. The graph of total dry weight of the roots determined at weekly intervals followed a typical S-shaped growth curve. The maximum dry weight of the crown roots was reached at approximately the silking stage in five of the strains and possibly the week following silking for the remaining two strains. It was believed that the inbred lines and hybrids might be expected to show relatively different degrees of resistance to lodging at successive stages of growth on the basis of the growth curves of their roots.

Striking differences were noted among the inbred lines in the ordinary development of lateral roots and in the amount of stimulated lateral root growth following injury of a main root by soil insects.

The top-to-root ratio was nearly 2:1 for some strains at 2 weeks after planting but varied from 12:1 to 23:1 among the strains at 15 weeks after planting. There was no close correlation among the inbred lines between the dry weights of the tops and of the roots.

The single-cross hybrids exceeded the inbred lines in dry weight of roots, dry weight of tops, diameter of main roots, length of roots, resistance to a vertical pull, diameter of culm, and plant height; they tended to approximate the inbred line with the greater number of main roots, but no consistent relation was evident with respect to the number of nodes with roots; they were intermediate between their constituent lines in the top to root ratio during most of the growing season. The double-cross hybrid and one of the single crosses (56×4–8) were nearly identical with respect to most of the root characters studied.

In the plants studied, about one additional whorl of crown roots was developed during each week of the first 8 or 9 weeks of the growing season.

The root systems of both the inbred lines and the hybrids were :xceedingly shallow as compared with those reported from the prairie egions of the United States. Data are given for the distribution of he main roots at three different periods during the growing season.

Within all strains the number of pounds required to pull a corn plant from the ground was most closely correlated with the dry weight of the crown roots.

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NUTRIENT VALUE OF THE PHOSPHORUS IN DEFLUORINATED PHOSPHATE, CALCIUM METAPHOSPHATE, AND OTHER PHOSPHATIC MATERIALS AS DETERMINED BY GROWTH OF PLANTS IN POT EXPERIMENTS [1]

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INTRODUCTION

In recent years much work has been done on the development of methods for the production of new phosphatic materials suitable for use as fertilizer. Some of these materials, particularly defluorinated phosphate, calcium metaphosphate, and potassium metaphosphate, offer considerable promise as sources of phosphorus for plant growth, either because of their high content of plant food and the consequent economy in transportation and handling costs or because of the possibility of effecting a considerable reduction in processing costs. In either case the primary object of the work is to supply the farmer with cheaper phosphate and at the same time to maintain at a high level the nutrient value of the phosphorus contained therein.

Defluorinated phosphate (prepared by heating phosphate rock at high temperatures in the presence of water vapor and silica) has been produced experimentally in two forms—calcined phosphate and fused phosphate rock—which differ in certain physical characteristics, but which appear to be identical in chemical structure (*28*).[4] Calcined phosphate, obtained in the form of a sintered or semifused, more or less porous clinker, is prepared by defluorinating phosphate rock at temperatures below the melting point of the charge, usually at 1,400° C. (*35, 46, 58, 59, 60*). Fused phosphate rock, obtained in the form of a hard glassy material, is prepared by defluorinating phosphate rock at temperatures above the melting point of the charge, usually at about 1,550° C. (*12*). Although these products are not produced commerically, they offer interesting possibilities as cheap sources of phosphorus for agricultural purposes, provided certain difficulties that have been encountered in attempts to produce them on a commercial scale can be overcome.

In addition to the adverse effect of incomplete volatilization of the fluorine (*7, 12, 32, 35, 46, 58, 59*), the citrate and citric acid solubilities of the phosphorus of defluorinated phosphate depend, within certain limits, on the particle size of the material (*12, 34*) and usually on the rate at which the product is cooled from the reaction temperature to atmospheric temperature (*47*). α-Tricalcium phosphate, silico carnotite (5 $CaO \cdot P_2O_5 \cdot SiO_2$), and a phase of unknown composition

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[1] Received for publication June 15, 1940.

[2] The pot experiments were made by R. P. Bartholomew, Arkansas Agricultural Experiment Station; R. L. Cook, Michigan Agricultural Experiment Station; D. W. Edwards, Hawaii Agricultural Experiment Station; W. T. McGeorge, Arizona Agricultural Experiment Station; W. H. Pierre, West Virginia Agricultural Experiment Station; and F. R. Reid, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] The Fertilizer Research Division was transferred to the Bureau of Plant Industry December 31, 1939.

[4] Italic numbers in parentheses refer to Literature Cited, p. 556.

are the principal constituents of quickly cooled defluorinated phosphates, whereas apatite usually appears as an important constituent in slowly cooled samples (*28*). The material, whether cooled quickly or slowly, contains little or no water-soluble phosphorus.

The researches of the Tennesse Valley Authority (*10, 11*) on calcium metaphosphate and those of the Bureau of Chemistry and Soils on potassium metaphosphate (*40, 41, 45*) have focused attention on the possibilities of these compounds as sources of phosphorus for plant growth, particularly because of their high content of nutrient elements and the consequent economy in handling and transportation costs. Calcium metaphosphate, as prepared by the Tennessee Valley Authority, is obtained by reacting phosphorus pentoxide with phosphate rock at a temperature of 1,200° C. and quickly cooling the product. Potassium metaphosphate can be obtained (1) by heating monopotassium orthophosphate at 700° C., (2) by reacting phosphoric acid with potassium chloride at temperatures up to 850° C., (3) by reacting phosphorus pentoxide and water vapor with potassium chloride, and (4) by smelting a mixture of phosphate rock, potassium silicate, and coke (*27, 55, 56, 64, 65*).

As reported in previous papers (*7, 32*), the results of greenhouse experiments indicated that quickly cooled, finely ground (80- or 100-mesh), low-fluorine calcined phosphate is as effective as superphosphate and dicalcium phosphate in promoting plant growth on soils having pH values below 7. The study reported in the present paper was undertaken to obtain information on the nutrient value of fused phosphate rock, calcium metaphosphate, reverted calcined phosphate, and, particularly, of different particle sizes of unreverted calcined phosphate. Experiments were also made with monocalcium phosphate, dicalcium phosphate, superphosphate, high-grade basic slag, Non-Acid Phosphate (prepared by heating phosphate rock with a potassium salt (*1*)), and ground phosphate rock.

The Arizona, Arkansas, Hawaii, Michigan, and West Virginia Agricultural Experiment Stations and the Bureau of Plant Industry, United States Department of Agriculture, cooperated in the study, the results of which are published in part in another paper (*62*). Although experiments were also made by several other State agricultural experiment stations, the results are not included herein, principally because either the soils did not respond to the phosphate applications, tests were run only on some of the phosphatic materials, or phosphorus determinations were not made on the harvested plants.

REVIEW OF LITERATURE

In agreement with the results previously cited (*7, 32*), Serralles (*67*) reported that quickly cooled, finely ground (80-mesh), low-fluorine (0.10 percent) calcined phosphate was as effective as superphosphate in pot experiments with oats on a phosphorus-deficient Morrison sandy loam soil (pH 6.2) from The Barrens near State College, Pa. Furthermore, the low-fluorine calcined phosphate, in which the citrate solubility of the phosphorus amounted to 92.6 percent, gave somewhat better results than material that contained 0.65 percent of fluorine and showed a 66.4-percent solubility of the phosphorus. Gilbert and Pember (*18*) also used the high-fluorine material in pot tests with barley, buckwheat, and millet on two

Bridgehampton very fine sandy loam soils (pH 4.5 and 6.0, respectively) from the farm of the Rhode Island Agricultural Experiment Station, Kingston, R. I. In general, the results agreed well with those obtained by the use of either monocalcium phosphate, dicalcium phosphate, or double superphosphate.[5] Excellent results have been obtained with calcined phosphate as a source of phosphorus for permanent pasture sod at the New Jersey Agricultural Experiment Station.[6] On the other hand, the results of plot tests by the Idaho station[7] indicate that calcined phosphate, though better than fused phosphate rock, is not as effective as double superphosphate in promoting the growth of alfalfa on alkaline soils. According to Taylor and Pierre,[8] calcined phosphate has a basic action on the soil, whereas ground phosphate rock and natural colloidal phosphate have very little effect in this respect.

In field experiments by the Montana Agricultural Experiment Station (*23*) with cereal crops and alfalfa on phosphorus-deficient alkaline soils, fused phosphate rock produced very small increases in yields as compared to those obtained by the use of water-soluble phosphates (monocalcium phosphate and double superphosphate). Dicalcium phosphate also gave poor results. Likewise, fused phosphate rock was a poor source of phosphorus for the growth of alfalfa and sugar beets in plot experiments by the Idaho station.[7] In a 2-year plot experiment on a Charlton loam soil (pH 5.2) at the Storrs (Connecticut) Agricultural Experiment Station (*6*), the average yield of U. S. Grade No. 1 white potatoes obtained by using fused phosphate rock as the source of phosphorus was 95 percent of that obtained by using double superphosphate. In field trials with corn, wheat, millet, cowpeas, soybeans, and potatoes on several phosphorus-deficient soils, Mooers of the Tennessee Agricultural Experiment Station (*51*) found that fused phosphate rock usually did not give as good results as either monocalcium phosphate, dicalcium phosphate, or double superphosphate. With monocalcium phosphate as 100, the average relative ratings of fused phosphate rock applied to these soils were 97 and 81 as determined by the Neubauer seedling-plant test and by the *Cunninghamella* test, respectively. The fused phosphate rock used in Mooers' work retained about 25 percent of the fluorine content of the original phosphate rock and the phosphorus therein was probably not more than 50 percent soluble in neutral ammonium citrate solution.

Several high-temperature forms of calcium metaphosphate, differing greatly in their chemical and physical properties, are known. According to Bartholomew and Jacob (*4*), calcium metaphosphate prepared by heating acid-free monocalcium phosphate to constant weight at 600° to 650° C. is insoluble in neutral ammonium citrate solution and practically insoluble in aqua regia, but is soluble in hot concentrated sulfuric acid. In greenhouse experiments, this compound, as well as calcium pyrophosphate prepared by heating pure dicalcium phosphate to constant weight at 800° C., had little or no value as a source of phosphorus for the growth of Sudan grass on Clarksville silt loam

[5] This material, which is made by treating phosphate rock with phosphoric acid, is also known as treble superphosphate, triple superphosphate, or concentrated superphosphate.

[6] SPRAGUE, H. B. Private communication.

[7] LARSON, H. W. E. Private communication.

[8] TAYLOR, J. R., JR., and PIERRE, W. H. THE VALUE OF DIFFERENT BASIC MATERIALS AND OF DOLOMITIC LIMESTONE OF DIFFERENT DEGREES OF FINENESS IN THE PRODUCTION OF NON-ACID-FORMING FERTILIZERS. Amer. Soc. Agron., Com. on Fert., Proc. 1935: 15–23, illus. 1935. [Mimeographed.]

(pH 5.7) at the Arkansas Agricultural Experiment Station (*4*) and for the growth of barley, red clover, and Japanese millet on two Bridgehampton very fine sandy loam soils (pH 4.35 and 6.03) at the Rhode Island station (*18*). At both stations, precipitated calcium pyrophosphate gave much better results than the material prepared by heating dicalcium phosphate. Jolibois and coworkers (*37*) report that calcium metaphosphate which had been crystallized at 500° C. was of no value as a source of phosphorus for the growth of spring barley in pot experiments on alkaline soil (pH 8.1) and of spring oats on acid soil (pH 4.9). Calcium metaphosphate which had been fused and quenched was 57 percent as effective as monocalcium phosphate in increasing the yield on the alkaline soil but was of no value on the acid soil. In pot experiments with several species of plants, Kida (*38, 39*) found that the fertilizing values of calcium, magnesium, iron, and aluminum metaphosphates and pyrophosphates, prepared by heating the orthophosphates, were lower than those of the respective orthophosphates.

The calcium metaphosphate produced by the Tennessee Valley Authority, by reacting phosphorus pentoxide with phosphate rock at 1,200° C. and quickly cooling the product, is almost completely soluble in neutral ammonium citrate solution according to the official method of analysis (*3, pp. 21–22*). Some of the properties of this product were studied by MacIntire, Hardin, and Oldham (*44*), who report that under parallel conditions suspensions of soil, subsoil, ferric oxide, and aluminum oxide in aqueous and ammonium citrate solutions of calcium metaphosphate and orthophosphate, respectively, absorbed decidedly larger quantities of metaphosphate radical (PO_3) than of orthophosphate radical (PO_4). According to Mooers (*51*), field trials with corn, wheat, millet, cowpeas, soybeans, and potatoes on a number of phosphorus-deficient soils in Tennessee showed no significant differences in effects on crop yields between monocalcium phosphate, double superphosphate, dicalcium phosphate, and calcium metaphosphate (prepared by the Tennessee Valley Authority) on the basis of pound for pound of total phosphorus applied. On the other hand, in a comparison of the Neubauer seedling-plant and the Cunninghamella tests as means of obtaining an index of the availabilty of the different phosphates for each of the five types of soil (Colbert, Decatur, Fullerton, Hartsells, and Montevallo), both tests gave ratings to calcium metaphosphate which were usually much lower than those obtained by the field trials. In a 2-year plot experiment on a Charlton loam soil (pH 5.2) at the Storrs (Conn.) Agricultural Experiment Station (*6*), the average yield of U. S. Grade No. 1 white potatoes obtained by using calcium metaphosphate (prepared by the Tennessee Valley Authority) as the source of phosphorus was 95.4 percent of that obtained by using double superphosphate and was practically the same as the yield obtained by using fused phosphate rock. In field trials by the Montana station (*23*), calcium metaphosphate, like fused phosphate rock, was an inefficient source of phosphorus for the growth of cereal crops and alfalfa on phosphorus-deficient alkaline soils. Likewise, calcium metaphosphate, though better than fused phosphate rock, was not, in general, as effective as double superphosphate in increasing the yields of alfalfa, sugar beets, and white potatoes in plot experiments by the Idaho station.[10]

The nutrient value of the phosphorus in alkali-metal metaphosphates and pyrophosphates has been investigated by several workers. Thus, Weissflog and Mengdehl (*74*) report that monopotassium orthophosphate, potassium metaphosphate and pyrophosphate, and calcium metaphosphate were about equally effective for the growth of corn in sterile culture solutions. Pure salts, the methods of preparation and properties of which are not stated, were used in the experiments. Although corn absorbed the metaphosphates and pyrophosphates, they were subsequently converted quickly into the orthophosphates, and their presence in the plant tissue could be detected only in the roots. In the culture solution itself, the conversion of the metaphosphates and pyrophosphates into the orthophosphates was accelerated by the growth of plants therein. Glixelli and Boratyński (*19*) found no differences in the yields of barley and wheat grown in water cultures containing orthophosphate, pyrophosphate, or metaphosphate, but the absorption of phosphorus was lower from metaphosphate than from either pyrophosphate or orthophosphate. Furthermore, salts of the highly polymerized metaphosphoric acid, prepared by treating the amorphous, difficultly volatile phosphorus pentoxide with water, were absorbed to a smaller degree than were salts of the less polymerized acid, obtained from the crystallized, easily volatile pentoxide.

Burgevin (*31*) conducted water-culture experiments with potassium metaphosphate and orthophosphate, in which vitiation of the results by transformation of metaphosphate into orthophosphate in the cultures was prevented by changing the solutions at 2-day intervals. Under these conditions the compounds were equally effective sources of phosphorus for the growth of corn. Also, he reports (*30*) that commercial potassium metaphosphate gave as good results as monopotassium orthophosphate in pot experiments with spring oats on a nearly neutral soil. When algae were grown in a nutrient solution containing monopotassium orthophosphate, Sommer and Booth (*68*) found that metaphosphate persisted in the living cells after orthophosphate and pyrophosphate could no longer be detected. The transformation of orthophosphate to metaphosphate and the persistence of the metaphosphate indicates that this form is important in the metabolic processes of algae.

In pot experiments on loamy sand and moorland soils (pH 4.4 to 6.5 in aqueous suspension), Krügel, Dreyspring, and Heinrich (*43*) found that Merck's sodium metaphosphate, which was readily soluble in water, contained 68.89 percent P_2O_5, and was probably the hexametaphosphate ($(NaPO_3)_6$), was practically as effective as an equivalent quantity of superphosphate in increasing the growth of winter wheat, yellow oats, winter oats, and winter barley. Bartholomew and Jacob (*4*) conducted pot experiments with potassium metaphosphate and pyrophosphate prepared by heating the pure monopotassium and dipotassium orthophosphates to constant weight at 810° to 820° and at 1,000° C., respectively. Both compounds were somewhat less effective than superphosphate in increasing the weight of Sudan grass grown on Clarksville silt loam (pH 5.7), but they were more effective than superphosphate in increasing the absorption of phosphorus by the plants. In pot experiments at the Rhode Island Agricultural Experiment Station (*18*), the sample of potassium metaphosphate was more effective than monocalcium phosphate in in-

hampton very fine sandy loam soils having pH values of 6.0 and 4.5, respectively. According to Kida (*38*), sodium metaphosphate and pyrophosphate, prepared by heating the corresponding orthophosphate, were as efficient as the orthophosphates as sources of phosphorus for barley, oats, and wheat grown in pots in Knop's culture medium.

Various processes have been proposed for the manufacture of available phosphates by heating phosphate rock with alkali-metal salts, usually the sulfates or carbonates of sodium and potassium, with or without the addition of silica, or silicates, and other reagents. These processes have been discussed by Fishburne (*15*), Guernsey and Yee (*24*), Messerschmitt (*48, 49*), and Waggaman and Easterwood (*73, pp. 120–132*). As yet, attempts to operate such processes on a commercial scale have not met with sustained success in this country, although the Non-Acid Fertilizer & Chemical Co. and its successor, the Kreiss Potassium Phosphate Co. (*1*), operated plants at Lakeland and later at Tampa, Fla., for several years prior to 1930. In Germany, however, a product known as Rhenania Phosphate (*16, 48, 49*) has been manufactured for more than 20 years by heating phosphate rock with alkali-metal salts under certain conditions. Similar products are manufactured in other European countries. For example, a material, formerly known as Vesta Phosphate (*21*), is produced in Belgium and sold under the name of Supraphosphate or Disintegrated Phosphate (*20, 42*); the same material is known in France as Basiphosphate (*8, 42*). Two products manufactured in Poland are known as Tomasyna and Supertomasyna (*42, 70*), respectively.

In this country Allison, Braham, and McMurtrey (*2*), Conner (*9*), Haskins (*25, 26*), Mooers (*50*), Ross, Jacob, and Beeson (*63*), and Thornton (*72*); in Australia, Teakle, Baron-Hay, and Thomas (*69*), and Thomas (*71*); in South Africa, Dodds (*13*); and in Europe, Erdély (*14*), Gerlach and Nolte (*17*), Graftiau (*20*), Graftiau and Courtoy (*21*), Graftiau, Giele, and Hardy (*22*), Krügel and Dreyspring (*42*), Niklas, Schropp, and Scharrer (*52*), Niklas, Strobel, and Scharrer (*53, 54*), Ràth (*57*), Terlikowski and Byczkowski (*70*), Wilhelmj (*75*), and Wilhelmj, Karst, and Gericke (*76*) have shown that citrate-soluble phosphates, prepared by heating phosphate rock with alkali salts, are usually excellent sources of phosphorus for plant growth.

MATERIALS AND EXPERIMENTAL METHODS

The particle size, and the phosphoric oxide (P_2O_5) and fluorine contents[11] of the phosphatic materials used in pot experiments are given in table 1. The phosphoric oxide insoluble in neutral ammonium citrate and 2-percent citric acid solutions, was determined by the official methods (*3, pp. 21–23, 36*). The citrate digestions, but not the citric acid digestions, were made in the presence of filter-paper pulp (*34*).

No. 1 is a commercial superphosphate prepared from Tennessee brown-rock phosphate. Nos. 2 and 3 (reagent quality salts) are monocalcium phosphate monohydrate and partially hydrated dicalcium phosphate, respectively.

Nos. 4 to 10, inclusive, represent different ranges of particle sizes of the same lot of calcined phosphate which was prepared on a semiworks scale by Darling & Co., East St. Louis, Ill., by heating Tennessee

[11] The chemical analyses were made by L. F. Rader, Jr., D. S. Reynolds, and T. H. Tremearne, Fertilizer

brown-rock phosphate in an oil-fired rotary kiln at 1,400° C. in the presence of water vapor. Nos. 4, 5, 6, and 7 were prepared by grinding separate quantities of the original calcined phosphate to the indicated degrees of fineness. In these samples (Nos. 4, 5, and 6), the distribution of particle size in the range 20 to 200 mesh is given in table 2.

TABLE 1.—*Phosphatic materials used in pot experiments*

No.	Material	Particle size		P_2O_5			Fluorine
				Total	Citrate-insoluble	Citric acid-insoluble	
		Mesh	*Millimeters*	*Percent*	*Percent*	*Percent*	*Percent*
1	Superphosphate	<40	<0.381	[1] 19.67	0.12	4.07	1.25
2	Monocalcium phosphate	<40	<.381	[2] 56.76	.00	.00	.00
3	Dicalcium phosphate	<40	<.381	48.53	2.77	.00	.00
4	Calcined phosphate	<20	<.833	37.40	7.80	4.74	.00
5	do	<40	<.381	37.36	4.72	3.06	.09
6	do	<80	<.175	37.18	3.73	2.77	.09
7	do	<200	<.074	37.20	3.68	2.78	.09
8	do	20–40	0.833–.381	37.30	9.53	5.29	.05
9	do	60–80	.221–.175	37.20	5.63	4.58	.15
10	do	100–150	.147–.104	37.05	4.46	3.44	.13
11	Calcined phosphate, reverted	<80	<.175	37.05	20.50	13.87	.09
12	Basic slag	<100	<.147	18.46	3.61	3.27	<.01
13	Calcium metaphosphate	<80	<.175	63.87	.24	43.83	.12
14	Fused phosphate rock	<80	<.175	28.97	4.60	2.40	.12
15	Non-Acid Phosphate [3]	<100	<.147	26.56	11.40	9.68	2.13
16	Tennessee brown-rock phosphate	<100	<.147	33.75	31.37	27.87	3.64

[1] Contained 12.74 percent of water-soluble P_2O_5.
[2] Contained 54.44 percent of water-soluble P_2O_5.
[3] Contained 2.71 percent of water-soluble K_2O.

TABLE 2.—*Distribution of particle size in calcined phosphates*

No.	Particle size	Distribution of particle size, mesh basis						
		20 to 40	40 to 60	60 to 80	80 to 100	100 to 150	150 to 200	200
	Mesh	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
4	<20	50.2	24.8	7.7	4.3	4.5	3.3	5.2
5	<40		25.7	17.3	10.5	11.3	9.7	25.5
6	<80				9.7	22.5	20.0	47.8

Nos. 8, 9, and 10 were obtained by sieving a quantity of the original calcined phosphate which had been ground to pass a 20-mesh sieve. In portions of these separates which were reground to pass a 200-mesh sieve in order to eliminate the effects of differences in particle size, the variations in the percentages of both citrate- and citric acid-insoluble phosphorus were comparatively small and, in keeping with the results of previous studies (*35, 46, 58, 59, 60*), were related to the percentages of fluorine in the respective samples (table 3).

As shown by Marshall and coworkers (*47*), defluorinated phosphates, including both calcined phosphates and fused phosphate rocks, which have been cooled rapidly from about 1,400° C. to room temperature are usually much more soluble in neutral ammonium citrate solution than are those which have been cooled slowly through this temperature range. Reversion of the phosphorus to the citrate-insoluble condition is usually much more pronounced in samples cooled in an atmosphere of water vapor than in those cooled in a dry atmosphere. The reverted

calcined phosphate (No. 11) was prepared by heating a portion of No. 6 for 1 hour at 1,000° C. in an atmosphere of water vapor. This treatment decreased the citrate solubility of the phosphorus from 90 to 44.7 percent, but had only a very slight effect on the content of total phosphorus; likewise, the citric acid solubility of the phosphorus decreased from 92.5 to 62.6 percent.

TABLE 3.—*Effect of particle size on solubility of calcined phosphate in neutral ammonium citrate and 2 percent citrate acid solutions*

No.	Particle size [1]	Total P_2O_5	Fluorine	Citrate-insoluble P_2O_5 in sample—		Citric acid-insoluble P_2O_5 in sample—	
				As used	Reground to <200 mesh	As used	Reground to <200 mesh
	Mesh	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
[2] 7	<200	37.20	0.09	3.68	3.68	2.78	2.78
8	20-40	37.30	.05	9.53	2.64	5.29	1.82
9	60-80	37.20	.15	5.63	4.92	4.58	4.36
10	100-150	37.05	.13	4.46	4.29	3.44	3.56

[1] Particle size of material as used in the pot experiments.
[2] Representative sample of the original calcined phosphate.

The basic slag (No. 12) is a high-grade imported material. The calcium metaphosphate (No. 13) and the fused phosphate rock (No. 14) were prepared by the Tennessee Valley Authority from Tennessee brown-rock phosphate by the processes described by Curtis and coworkers (*10, 11, 12*). No. 15 (Non-Acid Phosphate) was manufactured in 1925 by the Non-Acid Fertilizer & Chemical Co., Lakeland, Fla., by heating Florida pebble phosphate with a potassium salt (*1*). The Tennessee brown-rock phosphate (No. 16) is a commercial material, sold for direct application to the soil. It was ground so fine that 100, 98, 92, and 86 percent of the material passed 100-, 200-, 300-, and 400-mesh sieves, respectively.

The conditions of the pot experiments are outlined in table 4. For a given series of experiments, the phosphates were applied on the basis of equal quantities of total phosphorus. The plants used in the experiments were German millet (*Setaria italica*), Sudan grass (*Sorghum vulgare* var. *sudanense*), and tomato (*Lycopersicum esculentum*).

TABLE 4.—*Conditions of pot experiments*

Cooperating organization	Soil			Fertilizer applied per pot					Crop [1]				Replications
	Type	pH	Air-dry weight per pot	P_2O_5	Nitrogen		K_2O [2]		Kind	Date of seeding	Plants per pot	Date of harvesting	
					Compound	Weight	Compound	Weight					
			Kilograms	*Grams*		*Grams*		*Grams*			*Number*		*Number*
rizona Experiment Station.	Calcareous sandy loam [3]	9.5	3.0	[4] 0.50	None		None		Tomato	Feb. 4	51-138	Apr. 15	2
rkansas Experiment Station, first series.	Clarksville silt loam [5]	5.4	9.0	[6] .27	$(NH_4)_2SO_4$, $NaNO_3$	[7] 0.169	KCl	0.56	Sudan grass	Apr. 9	12	June 8	3
rkansas Experiment Station, second series.	do.[8]	7.1	9.0	[6] .27	do	[7] .169	KCl	.56	do	do	12	do	3
ureau of Plant Industry	Norfolk loamy fine sand	5.8	5.0	[9] .60	do	[10] .25	K_2SO_4	[11] .30	German millet	Jan. 21	10	Mar. 24	3
awaii Experiment Station	Dark reddish-brown friable clay.	5.2	5.0	[9] 4.50	NH_4NO_3	1.10	K_2SO_4	1.50	Sudan grass	June 9	40	Sept. 3	2
lichigan Experiment Station.	Miami	5.3	4.0	[4] .152	$(NH_4)_2SO_4$ [12]	.038	KCl [12]	[11] .038	do	Mar. 5	10	May 28	4
Vest Virginia Experiment Station, first series.	Dekalb silty clay loam [13]	5.3	7.0	[14] .35	$NaNO_3$, urea	[15] .42	KCl	.44	do	May 11	6	July 16	3
Vest Virginia Experiment Station, second series.	do.[13]	6.1	7.0	[14] .35	do	[15] .42	KCl	.44	do	do	6	do	3

[1] All crops were planted and harvested in 1930.
[2] Excluding K_2O applied in the form of Non-Acid Phosphate, except as indicated.
[3] Semiarid soil, very low in organic matter and containing 3 percent of $CaCO_3$.
[4] Phosphate was thoroughly mixed with the soil.
[5] The soil had been neither fertilized nor limed since 1926.
[6] All fertilizers were thoroughly mixed with the soil on April 3.
[7] 0.097 gm. from $(NH_4)_2SO_4$ and 0.072 gm. from $NaNO_3$.
[8] The soil had been neither fertilized nor limed for at least 18 years.
[9] All fertilizers were thoroughly mixed with the soil just before seeding.
[10] Two-thirds from $(NH_4)_2SO_4$ and one-third from $NaNO_3$.
[11] Including water-soluble K_2O applied in the form of Non-Acid Phosphate.
[12] Applied in solution to the surface of the soil.
[13] The soil had not been limed for 8 years.
[14] Phosphatic and potassic fertilizers were thoroughly mixed with the soil just before seeding.
[15] Equally *divided between* $NaNO_3$ and urea; 0.35 gm. of nitrogen was thoroughly mixed with the soil just before seeding and 0.07 gm. was applied as a top dressing on July 2.

EFFECT OF PHOSPHATIC MATERIALS ON DRY WEIGHT AND PHOSPHORUS CONTENT OF PLANTS

The average dry weight and phosphorus content of, and recoveries f applied phosphorus in, the plants grown on acid and neutral soils re given in table 5.[12] Data on the tomato plants grown by the rizona station on a calcareous alkaline soil (pH 9.5) are given in able 6.

'ABLE 5.—*Average dry weight and P_2O_5 content of plants and recovery of applied P_2O_5 (acid and neutral soils)*

DRY WEIGHT [1]

Phosphate treatment		German millet, Bureau of Plant Industry [2]	Sudan grass					
			Arkansas Experiment Station [3]		Hawaii Experiment Station [7]	Michigan Experiment Station [8]	West Virginia Experiment Station [4]	
o.	Materia		First series [5]	Second series [6]			First series [9]	Second series [10]
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
	No phosphorus	4.2	20.1	11.1	116.4	15.7	12.6	6.6
1	Superphosphate	7.6	24.2	20.0	201.1	23.7	22.7	22.8
2	Monocalcium phosphate	6.8	23.7	19.4	152.1	23.8	22.6	21.6
3	Dicalcium phosphate	8.3	24.9	20.9	188.8	24.7	23.2	23.1
4	Calcined phosphate, <20 mesh	6.3	21.9	18.0	193.6	22.8	21.8	20.4
5	Calcined phosphate, <40 mesh	7.1	24.0	18.0	197.8	24.5	23.5	24.4
6	Calcined phosphate, <80 mesh	7.2	24.0	21.1	194.2	22.2	25.0	23.4
7	Calcined phosphate, <200 mesh	7.8	25.8	20.0	184.9	21.2	24.5	23.7
8	Calcined phosphate, 20 to 40 mesh	6.0	23.6	17.7	173.8	17.9	18.6	18.3
9	Calcined phosphate, 60 to 80 mesh	8.4	21.6	19.2	189.7	23.0	24.6	21.2
0	Calcined phosphate, 100 to 150 mesh	8.7	23.4	20.7	187.3	22.8	23.3	24.5
1	Calcined phosphate, reverted	7.0	22.4	20.7	182.2	22.0	25.2	20.8
2	Basic slag	7.0	23.4	21.7	189.8	19.8	24.0	22.4
3	Calcium metaphosphate	8.1	22.6	21.8	173.2	22.7	25.1	24.5
4	Fused phosphate rock	8.6	23.3	21.3	187.6	21.4	25.7	24.2
5	Non-Acid Phosphate	8.3	23.6	20.8	173.1	21.9	23.7	21.9
6	Tennessee brown-rock phosphate	3.5	20.9	16.3	148.6	19.8	17.8	10.2

P_2O_5 CONTENT [1]

	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams
No phosphorus	22.1	52.3	34.4	105.9	34.9	34.5	20.9
Superphosphate	50.5	59.8	51.2	396.2	58.5	60.6	70.9
Monocalcium phosphate	49.8	57.8	49.7	237.3	58.1	65.8	70.1
Dicalcium phosphate	64.6	61.5	61.6	390.8	59.5	74.0	66.5
Calcined phosphate, <20 mesh	34.6	53.4	50.8	449.2	57.0	65.4	65.5
Calcined phosphate, <40 mesh	43.9	61.4	49.5	395.6	52.9	84.4	64.7
Calcined phosphate, <80 mesh	47.8	60.1	54.9	462.2	50.8	77.3	80.5
Calcined phosphate, <200 mesh	63.6	66.6	57.4	425.3	53.6	68.1	71.1
Calcined phosphate, 20 to 40 mesh	32.9	62.1	52.7	469.3	43.7	53.6	61.1
Calcined phosphate, 60 to 80 mesh	48.0	58.7	55.9	510.3	53.1	71.3	78.0
Calcined phosphate, 100 to 150 mesh	53.8	66.7	55.9	509.5	52.0	82.7	82.8
Calcined phosphate, reverted	33.7	63.8	65.7	353.5	49.5	74.3	62.4
Basic slag	41.7	69.5	65.8	432.7	46.6	76.8	63.8
Calcium metaphosphate	59.3	63.7	52.5	389.7	50.4	80.8	74.2
Fused phosphate rock	55.1	63.6	51.3	474.6	47.5	82.5	76.0
Non-Acid Phosphate	41.8	64.2	54.9	249.3	46.0	62.6	56.1
Tennessee brown-rock phosphate	16.0	55.0	46.8	148.6	42.2	51.3	33.7

Per pot; aerial portions of plants only.
Total P_2O_5 applied at rate of 0.60 gm. per pot (240 pounds per acre); pH of soil 5.8.
Total P_2O_5 applied at rate of 0.27 gm. per pot (60 pounds per acre).
Total P_2O_5 applied at rate of 0.35 gm. per pot (100 pounds per acre).
pH of soil 5.4.
pH of soil 7.1.
Total P_2O_5 applied at rate of 4.5 gm. per pot (1,300 pounds per acre); pH of soil 5.2.
Total P_2O_5 applied at rate of 0.152 gm. per pot (76 pounds per acre); pH of soil 5.3.
pH of soil 5.3.
pH of soil 6.1.

TABLE 5.—*Average dry weight and P_2O_5 content of plants and recovery of applied P_2O_5 (acid and neutral soils)*—Continued

RECOVERY OF APPLIED P_2O_5[1]

Phosphate treatment		German millet, Bureau of Plant Industry	Sudan grass					
No.	Material		Arkansas Experiment Station		Hawaii Experiment Station	Michigan Experiment Station	West Virginia Experiment Station	
			First series	Second series			First series	Second series
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	Superphosphate	4.7	2.7	6.2	6.5	15.5	7.5	14.3
2	Monocalcium phosphate	4.6	2.0	5.7	2.9	15.3	8.9	14.1
3	Dicalcium phosphate	7.1	3.4	6.4	6.3	16.2	11.3	13.0
4	Calcined phosphate, <20 mesh	2.1	.4	6.1	7.6	14.5	8.8	12.7
5	Calcined phosphate, <40 mesh	3.6	3.4	5.6	6.4	11.8	14.3	12.5
6	Calcined phosphate, <80 mesh	4.3	6.2	7.6	7.9	10.5	12.3	17.0
7	Calcined phosphate, <200 mesh	5.3	6.0	8.5	7.1	12.3	9.6	14.3
8	Calcined phosphate, 20 to 40 mesh	1.8	3.6	6.8	8.1	5.8	5.5	11.5
9	Calcined phosphate, 60 to 80 mesh	4.3	2.4	8.0	9.0	12.0	10.5	16.3
10	Calcined phosphate, 100 to 150 mesh	5.3	5.3	7.7	9.0	11.3	13.8	17.7
11	Calcined phosphate, reverted	1.9	4.3	7.9	5.5	9.6	11.4	11.9
12	Basic slag	3.3	6.4	7.9	7.3	7.7	12.1	12.3
13	Calcium metaphosphate	6.2	4.2	6.7	6.3	10.2	13.2	15.2
14	Fused phosphate rock	5.5	4.2	6.3	8.2	8.3	13.7	15.7
15	Non-Acid Phosphate	3.3	4.4	7.6	3.0	7.3	8.0	10.1
16	Tennessee brown-rock phosphate	−1.0	1.0	4.6	.9	4.8	4.8	3.7

[1] Per pot; aerial portions of plant only.

TABLE 6.—*Effect of phosphatic materials on tomato plants grown on soil having a pH value of 9.5* [1]

No.	Material	Plants per pot	Color of plants	Condition of plants	Dry weight per 100 plants	P_2O_5 content per 100 plants	Efficiency of phosphatic material as indicated by increase in—	
							Dry weight of 100 plants[2]	P_2O_5 content of 100 plants[2]
		Number			*Grams*	*Milligrams*		
	No phosphorus	122	Purple	Poor	4.1	17.8	----	----
1	Superphosphate	80	Green	Good	9.3	75.4	100	100
2	Monocalcium phosphate	51	do	do	11.0	93.5	133	131
3	Dicalcium phosphate	74	Purple	Poor	7.1	41.9	58	42
4	Calcined phosphate, <20 mesh	87	do	do	8.4	42.1	83	42
5	Calcined phosphate, <40 mesh	88	do	do	7.6	51.6	67	59
6	Calcined phosphate, <80 mesh	87	do	do	7.8	35.2	71	30
7	Calcined phosphate, <200 mesh	81	do	do	9.8	48.9	110	54
8	Calcined phosphate, 20 to 40 mesh	138	do	do	8.2	27.8	79	17
9	Calcined phosphate, 60 to 80 mesh	101	do	do	5.9	21.5	35	6
10	Calcined phosphate, 100 to 150 mesh.	88	do	do	4.8	24.0	13	11
11	Calcined phosphate, reverted	109	do	do	5.4	23.6	25	10
12	Basic slag	102	do	do	6.5	38.4	46	36
13	Calcium metaphosphate	69	do	do	6.7	74.5	50	98
14	Fused phosphate rock	130	do	do	5.4	30.7	25	22
15	Non-Acid Phosphate	91	do	do	7.7	57.6	69	69
16	Tennessee brown-rock phosphate	91	do	Very poor.	4.0	27.2	−2	16

[1] Experiments by Arizona Agricultural Experiment Station on a semiarid, sandy loam soil that was very low in organic matter and contained 3 percent of $CaCO_3$.
[2] Based on the increase from superphosphate as 100.

In the acid and neutral soils (table 5), marked increases in the growth and the phosphorus content of the plants were nearly always obtained with all the chemically processed phosphates. Although it

rials in a given series of experiments, increases in the dry weight anp the phosphorus content of the plants were also obtained by the application of ground phosphate rock (No. 16), except in the experiments by the Bureau of Plant Industry.

A fair comparison of the relative effects of the phosphates applied to the alkaline soil (table 6) cannot be obtained, because the number of plants grown per pot varied widely. In agreement with investigations by the Idaho [13] and Montana (*23*) Agricultural Experiment Stations, the data indicate, nevertheless, that the so-called water-insoluble phosphates are not good sources of phosphorus for the growth of plants on alkaline soil. The results are said to be in accord with the usual experience with Arizona soils.[14] It will be noted that the plants which received calcium metaphosphate (No. 13) showed a high absorption of phosphorus, but this was not accompanied by any marked improvement in their condition and color. Because of the lack of uniformity in the experimental procedure, this series of experiments will be excluded from further discussion.

In previous tests comparing calcined phosphates, which varied widely in fluorine content and likewise in ammonium citrate- and citric acid-soluble phosphorus, the effect of the materials on the phosphorus content of the plants in a given series of experiments was, with a few exceptions, in the same order as their effect on the dry weight (*32*). When all the samples used in the present tests (table 5) are considered on the basis of the increase in the dry weight of the plants, the relative order of the phosphatic materials in a given series of experiments in many instances differs rather widely from that based on the phosphorus content of the plants. If, however, the samples involving the different ranges of particle size of the calcined phosphate (Nos. 4, 5, 7, 8, 9, and 10) are excluded, the agreement is very much better, and both bases of comparison usually place the materials in either the same or nearly the same relative order.

Scarseth and Chandler (*66*) studied the residual phosphate situation with a nearly level Norfolk loamy sand in Alabama which had been used for a 26-year period in an experiment involving a 3-year rotation of cotton, corn, and oats with various legumes and with different phosphatic fertilizer treatments. Where superphosphate was used, 32 percent of the applied phosphorus was utilized by the plants, 8 percent remained in the soil, and 60 percent was carried away with the clay fractions lost by erosion. With ground phosphate rock the corresponding figures were 9, 9, and 82, percent, respectively. As indicated by the phosphorus content of the aerial parts of the plants, the recovery of the applied phosphorus in the present experiments usually did not exceed 10 percent, and in no case was it as high as 20 percent (table 5). With one exception (calcined phosphate No. 4, Arkansas station, first series), the recoveries of phosphorus from ground phosphate rock (No. 16) were lower, usually very much lower, than those from the other materials. With the same type of soil and the same weight of applied phosphorus, the recoveries of phosphorus from all the materials, except ground phosphate rock at the West Virginia station, increased with the pH value of the soil (Arkansas and West Virginia stations). Contrary to the results of previous

[13] See footnote 7, p. 541.
[14] McGeorge, W. T. Private communication. Arizona Agricultural Experiment Station

experiments (*32*), there was no relation between the recovery and the per acre rate of application of the phosphorus.

RELATIVE EFFICIENCY OF PHOSPHATIC MATERIALS

The relative efficiency of the different types of phosphates, as indicated by the increase in the dry weight and phosphorus content of plants grown on acid and neutral soils, is shown in figure 1, and similar data for the different ranges of particle sizes of the calcined phosphate (Nos. 4 to 10, inclusive) are shown in figure 2. The efficiency of the phosphates is based upon 100 as the increase in dry weight or phosphorus content due to the application of superphosphate. The Bureau of Plant Industry used German millet as the test crop; the experiment stations used Sudan grass. In figure 1, the materials differ not only in the chemical nature of the phosphorus-bearing compound or compounds but usually in their content of calcium and other elements, as well as in their basicities and hence in their ability to neutralize soil acids. Therefore, the changes in the plant dry weights and phosphorus content obtained with these materials do not necessarily represent merely the specific effects of the applied phosphorus. In figure 2, on the other hand, the compared materials are practically identical in chemical composition, and the differences in their efficiency can be attributed entirely to particle-size effects.

Although for a given material the efficiencies based on the dry weight of the plants show rather wide variations in some instances, they are usually much more consistent than those based on the phosphorus content. Furthermore, for a given material in a given series of experiments, the efficiency based on the dry weight is usually lower, often very much lower, than that based on the phosphorus content, which is contrary to the results of previous experiments (*32*).

On the basis of the dry-weight increases, the average relative efficiency of the different types of phosphatic materials (fig. 1) falls in the decreasing order: Dicalcium phosphate, 107; fused phosphate rock, 102; superphosphate, 100; $<$80-mesh calcined phosphate, 99; calcium metaphosphate, 98; Non-Acid Phosphate, 95; basic slag, 90; reverted calcined phosphate, 88; monocalcium phosphate, 84; and Tennessee brown-rock phosphate, 31. On the basis of the increases in phosphorus content of the plants, the order is: $<$80-mesh calcined phosphate, 130; fused phosphate rock and calcium metaphosphate, 120; basic slag, 119; dicalcium phosphate, 117; superphosphate and reverted calcined phosphate, 100; monocalcium phosphate and Non-Acid Phosphate, 89; and Tennessee brown-rock phosphate, 32. Although the two bases of comparison do not place the efficiencies of the materials (except those of fused phosphate rock and ground phosphate rock) in the same order, both bases give high ratings to dicalcium phosphate, fused phosphate rock, $<$80-mesh calcined phosphate, and calcium metaphosphate and a comparatively low rating to ground phosphate rock.

In nearly all the tests dicalcium phosphate was equal or superior to superphosphate as a source of phosphorous for the growth of plants on acid and neutral soils. Good results were also obtained with this material in previous experiments (*32, 61, 63*). On the other hand, monocalcium phosphate was usually less efficient than superphosphate (fig. 1). In view of the high average efficiency of the dicalcium phos-

phate, <80-mesh calcined phosphate, fused phosphate rock, and calcium metaphosphate (materials which are either very low in or free from sulfur) as compared with that of the sulfur-rich superphosphate,

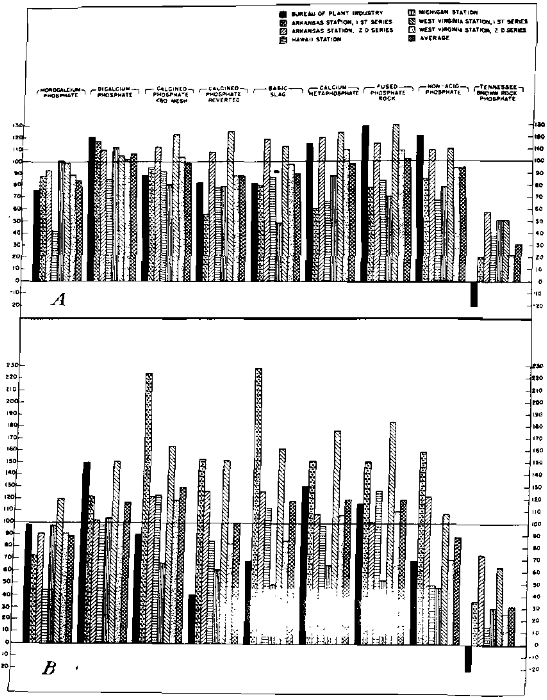

FIGURE 1.—Relative efficiency of different types of phosphatic materials, as indicated by increase in dry weight and phosphorus content of plants grown on acid and neutral soils, based on the increase from superphosphate as 100: *A*, Efficiency of phosphate as indicated by increase in dry weight; *B*, efficiency of phosphate as indicated by increase in phosphorus content of plants.

he relatively low efficiency of the monocalcium phosphate cannot b ttributed to the absence of sulfur therefrom.

As shown in figure 1, the average efficiency of the Non-Acid Phos-hate in increasing the dry weight of the plants was slightly highe

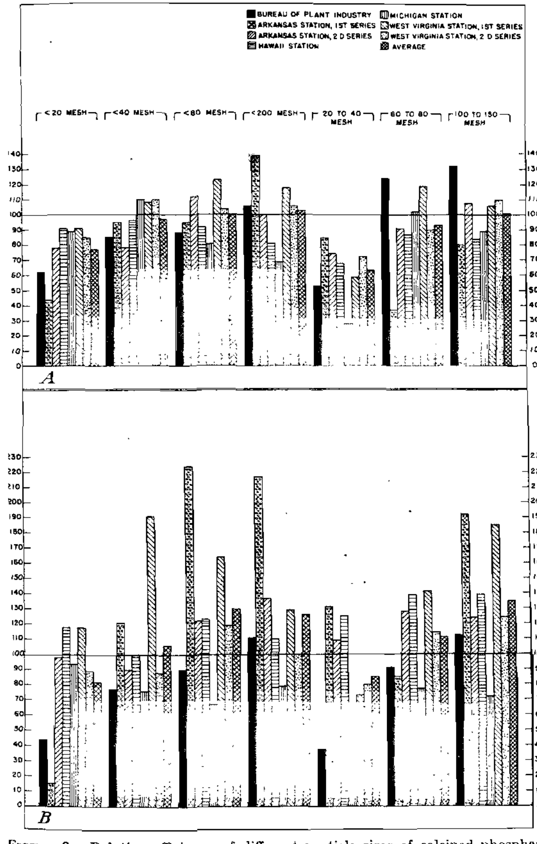

FIGURE 2.—Relative efficiency of different particle sizes of calcined phosphate as indicated by increase in dry weight and phosphorus content of plants grown on acid and neutral soils, based on the increase from superphosphate as 100: *A*, Efficiency of phosphate as indicated by increase in dry weight of plants; *B*, efficiency of phosphate as indicated by increase in phosphorus content of

than that of the imported basic slag. In other experiments with the same materials (*63*),[15] the efficiency was in the reverse order. In tests by the Neubauer seedling-plant method, Thornton (*72*) [15] found that Non-Acid Phosphate, applied to an acid silt loam soil (pH 5.4), was 79 to 85 percent as effective as monocalcium phosphate. The average efficiency of reverted calcined phosphate was significantly lower than that of the <80-mesh unreverted material from which it was prepared (fig. 1).

Although the results for a given material vary rather widely in some cases, the average efficiency (fig. 2) shows clearly the effect of the particle size on the fertilizing value of calcined phosphate. Thus, the average efficiency of the material increased markedly as the fineness of the particles was increased from 100 percent through a 20-mesh sieve to 100 percent through a 40-mesh sieve, and to a smaller extent as the particle size was decreased still further. Likewise, 20- to 40-mesh particles were, in general, considerably less efficient than were 60- to 80-mesh particles, and the latter were somewhat less efficient than 100- to 150-mesh material. It appears, however, that little is gained by increasing the fineness of calcined phosphate beyond 80 mesh. The average efficiency of the 80-mesh product was somewhat lower than that of similar material used in previous experiments (*32*).

COMPARATIVE EFFICIENCY AND SOLUBILITY OF PHOSPHATIC MATERIALS

The average relative efficiency of most of the materials, as indicated by the increases in the dry weights of plants grown on acid and neutral soils, was higher than would be expected on the basis of their solubility in neutral ammonium citrate solution (table 7). In particular, the average responses to applications of reverted calcined phosphate and of Non-Acid Phosphate were very much higher than the respective citrate solubilities of the phosphorus contained therein. On the other hand, the average efficiencies (indicated by increases in dry weights) of the monocalcium phosphate, <20-mesh calcined phosphate, 20- to 40-mesh calcined phosphate, and calcium metaphosphate were somewhat lower than the respective citrate solubilities of the phosphorus. The average efficiency of the different ranges of particle sizes of the calcined phosphate (Nos. 4 to 10, inclusive) was usually in the same order as the solubility of the phosphorus in either neutral ammonium citrate or 2-percent citric acid solution.

Aside from the monocalcium phosphate, which was completely soluble in either solvent, all but two of the materials (superphosphate and calcium metaphosphate) showed higher solubility of the phosphorus in citric acid than in ammonium citrate. Previous studies (*4, 5, 29, 33*) have shown that iron and aluminum orthophosphates are more soluble in neutral ammonium citrate solution than in 2-percent citric acid, whereas the reverse is true of the calcium orthophosphates (*32, 33, 36*). As compared to the citrate solubility, the lower citric acid solubility of the phosphorus in superphosphate is no doubt due to the presence of iron or aluminum phosphates, or both. Since the conditions under which the sample is treated for the determination of citrate-soluble phosphorus (*3, pp. 21–22*) are more favorable for the hydrolysis of metaphosphate to orthophosphate than those in the determination of citric acid-soluble phosphorus (*3, p. 36*), the greater solubility of the calcium metaphosphate in the first solvent

is probably due principally to a higher conversion into orthophosphate during the digestion of the sample and the filtration of the extract.

TABLE 7.—*Comparative efficiency and solubility of phosphatic materials*

No.	Material	Average efficiency of phosphatic material as indicated by increase in—		Solubility of phosphatic material as determined by—	
		Dry weight of plants [1]	Phosphorus content of plants [1]	Neutral ammonium citrate method [2]	2-percent citric acid method [2]
				Percent	*Percent*
1	Superphosphate	100	100	99.4	79.3
2	Monocalcium phosphate	84	89	100.0	100.0
3	Dicalcium phosphate	107	117	94.3	100.0
4	Calcined phosphate, <20 mesh	77	82	79.1	87.3
5	Calcined phosphate. <40 mesh	97	106	87.4	91.8
6	Calcined phosphate, <80 mesh	99	130	90.0	92.5
7	Calcined phosphate, <200 mesh	103	126	90.1	92.5
8	Calcined phosphate, 20 to 40 mesh	63	85	74.5	85.8
9	Calcined phosphate, 60 to 80 mesh	93	111	84.9	87.7
10	Calcined phosphate, 100 to 150 mesh	101	135	88.0	90.7
11	Calcined phosphate, reverted	88	100	44.7	62.6
12	Basic slag	90	119	80.4	82.3
13	Calcium metaphosphate	98	120	99.6	31.4
14	Fused phosphate rock	102	120	84.1	91.4
15	Non-Acid Phosphate	95	89	57.1	63.6
16	Tennessee brown-rock phosphate	31	32	7.1	17.4

[1] Based on the increase from superphosphate as 100, excluding results obtained with alkaline soil by the Arizona Agricultural Experiment Station.
[2] Based on the total phosphorus content of the material.

All the experiments with calcium metaphosphate (fig. 1 and table 7) show that its value as a source of phosphorus for the growth of plants on acid and neutral soils is correlated much more closely with the solubility of the phosphorus in neutral ammonium citrate solution than in 2-percent citric acid. In agreement with the results of previous studies (*32*, *36*), on the other hand, the citric-acid method seems to give a better index of the values of finely ground calcium orthophosphates as sources of phosphorus for the growth of plants on such soils than does the neutral ammonium citrate method. Although the evidence at hand is insufficient to justify a definite conclusion, it appears that neither method is satisfactory for the evaluation of phosphates for application to alkaline soil (table 6) and that water-soluble phosphates are the best sources of phosphorus for plant growth on such soil.

SUMMARY

In order to obtain information on the nutrient value of the phosphorus in defluorinated phosphates (calcined phosphate and fused phosphate rock) and the high-temperature form of calcium metaphosphate, pot experiments were made with Sudan grass and German millet on Clarksville silt loam, Norfolk loamy fine sand, Miami soil, Dekalb silty clay loam, and a dark reddish-brown friable clay, ranging in pH from 5.2 to 7.1, and with tomatoes on a calcareous sandy loam of pH 9.5. Particular attention was given to the nutrient value of calcined phosphate as affected by the particle size of the material. Experiments were made with reverted calcined phosphate in which a considerable portion of the phosphorus had been converted into

and 2-percent citric acid solutions by heating the soluble material at 1,000° C. in the presence of water vapor. Superpbosphate, monocalcium phosphate, dicalcium phosphate, high-grade basic slag, ground raw phosphate rock, and Non-Acid Phosphate (prepared by heating a mixture of phosphate rock and a potassium salt at high temperature) were also included in the tests.

In general, finely ground unreverted calcined phosphate (<80 mesh, or finer), fused phosphate rock, and calcium metaphosphate were approximately equal to superphosphate as sources of phosphorus for the growth of plants on acid and neutral soils, but the results of a single series of experiments indicated that these materials, as well as the other types of water-insoluble phosphates, are not so effective as water-soluble phosphates (monocalcium phosphate and superphosphate) on alkaline soils.

The average efficiency of the phosphorus of unreverted calcined phosphate increased markedly as the fineness of the particles was increased from 100 percent through a 20-mesh sieve to 100 percent through a 40-mesh sieve, and to a smaller extent as the particle size was decreased still further. Likewise, 20- to 40-mesh particles were, in general, considerably less efficient than 60- to 80-mesh particles, and the latter were somewhat less efficient than 100- to 150-mesh material. It appears, however, that little is gained by increasing the fineness of calcined phosphate beyond 80 mesh.

The average efficiency of reverted calcined phosphate and Non-Acid Phosphate in increasing the growth of plants on acid and neutral soils was much higher than would be expected on the basis of the citrate and citric acid solubility of the phosphorus contained therein. The average efficiency of calcium metaphosphate was much higher than its solubility in 2-percent citric acid indicated, but was in good agreement with its solubility in neutral ammonium citrate solution. With this material, superphosphate, and the coarsely ground unreverted calcined phosphate (<20-mesh and 20- to 40-mesh materials) as exceptions, the solubility of the phosphorus in 2-percent citric acid appeared, in general, to give a somewhat better index of the values of the phosphatic materials for the growth of plants on acid and neutral soils than did the solubility in neutral ammonium citrate.

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ESTIMATES OF PRODUCING ABILITY IN DAIRY CATTLE[1]

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INTRODUCTION

Progress in improving dairy cattle through selective breeding based on production performance is basically determined by the accuracy with which production records indicate inherited differences in producing ability. This accuracy becomes greater with any reduction in the variation caused by nonhereditary influences. Recent expansion in the use of production records for dairy cattle improvement, and the present variety in the kinds of production records and adjustments for developmental or management influences used, indicate the desirability of knowing whether any one kind of record has advantages over the others and which adjustments for nonhereditary influences on production records contribute materially to their accuracy. While numerous investigators have studied the environmental and developmental influences on production and have derived factors for standardizing records for such influences, only limited attention has been given to the relative genetic worth of corrected and raw records, and of the different kinds of production records. In this study the degree of inherency of five kinds of production records, before and after each correction for nonhereditary influences, is determined among the records of the same cows to see which estimate of producing ability is of greatest practical usefulness for selection purposes.

SOURCE OF DATA

The data include 1,574 lactation and 1,456 testing-year records of 274 Holsteins from 41 herds. Special fieldmen obtained the desired records directly from Dairy Herd Improvement Association herdbooks at the farms in connection with herd studies sponsored by the Germ Plasm Survey of the United States Department of Agriculture in 1935. The cows selected for this study were tested during at least their first five lactations and had no recorded mastitis, injury, or abortion, during this time. Testing-year records were discarded for years in which a cow freshened for the first time later than the third month or left the herd before the tenth month. Actual yields were used except

[1] Received for publication January 8, 1940. Paper from the Department of Genetics (No. 255), Wisconsin Agricultural Experiment Station. Some assistance was provided by the National Youth Administration and the Work Projects Administration during the course of the investigation.

[2] The writer wishes to express his appreciation to Dr. L. J. Cole at whose suggestion the work was initiated, and to Drs. A. B. Chapman and L. E. Casida, of the Wisconsin Station, and Dr. J. L. Lush, of the Iowa Station, for many helpful suggestions and for criticism of the manuscript.

for some three and four milkings-per-day records in a few Holste herds, and these were corrected to a two-daily-milkings basis wi Norton's factors (*23*).[3]

METHOD OF INVESTIGATION

Each cow's lifetime production was calculated in terms of five kin of records (240-day, 305-day, 365-day, total lactation, and testin year), making it possible to compare the different kinds of recor within the same cows and under the same environmental condition The means and standard errors for the five kinds of productic records and the factors affecting yield are as follows:

Record or factor		*Mean*
First 240-day butterfat yield	pounds	318 ± 1.
First 305-day butterfat yield	do	362 ± 2.
First 365-day butterfat yield	do	380 ± 2.
Total lactation period butterfat yield	do	389 ± 2.
Calving interval [1]	days	390 ± 1.
Lactation period length	do	340 ± 1.
Dry period length [1]	weeks	6. 9 ± 0.
Testing-year butterfat yield	pounds	375 ± 2.
Period milked during the testing year	days	322 ± 0.

[1] In some cases the calving interval and following dry period were unknown for the last lactation. The fore, these means were based on only 1,447 records instead of 1,574.

The measure of usefulness of production records in selecting breedir stock which was employed to determine the value of adjustment fc environmental influences, and to compare the five kinds of recorc before and after such adjustments, was the average within-her correlation between records of the same cow. This may be terme "repeatability," since it indicates the degree to which records of th same cow repeat themselves (i. e., tend to be more like each othe than they are like records of other cows in the same herd). Th measure had previously been used by Lush and his coworkers (*20, 21* It is calculated by the analysis of variance method of Fisher (*8*), i which the ratio of the between-class to the total variance may b expressed either as the portion of the variance due to difference between the classes (e. g., those due to herd differences, or to co differences within herds), or as the average correlation betwee observations in the same class (e. g., between records in the sam herd, or records of the same cow).

Smaller differences in repeatability are statistically significant whe the two variables compared are highly correlated with each other, a in the present study (e. g., the actual and age-corrected records, o the 305-day and 365-day records, for the same lactations), than whe two more or less independent variables are compared (e. g., 305-da records in two different breeds, or comparing production in cattle wit litter size in swine).

The relative importance of the various sources of environment variation in production, and the degree of repeatability or inherenc of each influence were determined by the analysis of variance metho Whenever the association of environmental influences with each othe or with production was essentially linear, the average correlation an regression among records of the same cow were obtained by th analysis of covariance (*8*) and used in calculating the percentag correction factors.

[3] Italic numbers in parentheses refer to Literature Cited, p. [illegible]

REPEATABILITY OF UNADJUSTED RECORDS

The repeatability of the production records was calculated on a within-herd basis, since the herd differences, which accounted for one-fifth to one-tenth of the total variation in production records (table 1), are greatly influenced by herd differences in management practices while variation between the lifetime averages of different cows in the same herd are more largely genetic in origin. The repeatability of the unadjusted records was obtained primarily for later use in deciding what had been gained by adjustment for nonhereditary influences, and varied from 0.23 for the 240-day to 0.26 for the total lactation records (table 1).

Differences between herds, and between cows in the same herd, accounted for significant[4] portions of the variation in calving interval, lactation length, days milked during the testing year, and dry period length (table 1). Inherent differences between cows in the same herd were most important in dry period length, which is associated with the persistency level of the cow more closely than are lactation length and days milked.

ADJUSTMENT FOR AGE

The data for age are unique in that every cow was on test from her first calving until past the age of maturity. Thus there was no possibility that the mature age records represented a selected group of cows, inherently higher in producing ability than those with immature records, as was the case with the data from which many of the 40 or more sets of age-correction factors have been derived by various workers. There is the possibility, however, that the influence of age in these data may not be entirely representative, if cows staying in the herd for five or more records tend to be those with relatively higher immature records, and if the percentage age change in yield varies with the level of inherent producing ability, as seems likely from these data (*7*) and from the work of Sanders (*28, 30*) and Schmidt (*32*). The average annual production of these cows was 375 pounds of fat, which was significantly greater (by 15 pounds of fat) than that of other cows in the same herds, and probably over 60 pounds above the average of all cows in tested herds.

Sanders (*28, 30*), Schmidt (*32*), Gaines and Palfrey (*14*), Kendrick,[5] Plum (*24*), and others have recognized the complicating effects of selection in their studies of age and have attempted to avoid these difficulties by studying the influence of age within individual animals. Tuff (*34*) and Ward and Campbell (*36*) have proposed a regression equation method of adjustment for age which also corrects for unknown temporary environmental influences on immature records, but allowance for this environmental regression toward the population average can best be made as suggested by Lush (*19*) in a separate calculation which takes into account the number of records for each cow and the individual herd average, and which treats immature and mature records alike.

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[4] "Significant" is used throughout to designate differences which would occur by chance alone less than 1 percent of the time; those with a probability of chance occurrence of 1 to 5 percent are termed "barely significant."

[5] KENDRICK, J. F., comp. TESTER'S COMPUTER. U. S. Bur. Dairy Indus., 22 pp. November 1937. [Mimeographed.] See pp. 17–19.

... herd and cow differences [a]

Source of variation	Variance symbols [3]	Degrees of freedom [4]	Variance in butterfat yield during lactation: First 240 days	First 305 days	First 365 days	Total lactation period	Variance in length of lactation period	Degrees of freedom [4]	Variance in—: Calving interval	Dry period	Degrees of freedom	Variance in testing year: Period milked	Butterfat yield
			Pounds	*Pounds*	*Pounds*	*Pounds*	*Days*		*Days*	*Weeks*		*Days*	*Pounds*
ds	H	(40)	736	1,180	1,266	1,058	255	(40)	388	2.53	(40)	103.6	1,500
ws within herds	C	(233)	1,068	1,426	2,000	2,676	578	(233)	364	3.75	(233)	86.6	1,533
ords of same cow	R	1,300	3,612	4,820	5,735	7,513	4,077	1,173	4,718	17.90	1,182	926.4	4,886
relation between records in same erd.	$\frac{H}{H+C+R}$		.136	.159	.141	.094	.052		.071	.105		.093	.189
aherd correlation between records same cow.	$\frac{C}{C+R}$		[5].228	[5].228	[5].259	[5].263	.124		.072	.173		.085	[5].239

Except for some records corrected for number of daily milkings.
In this and following tables all values are significant ($P<0.01$) unless otherwise indi-d; * indicates barely significant values ($P>0.01<0.05$); ** indicates nonsignificant es ($P>0.05$).

Total mean square $=R+\frac{n_c-1}{N-1}\cdot\frac{N}{n_c}\cdot C+\frac{n_h-1}{N-1}\cdot\frac{N}{n_h}\cdot H=\left[\Sigma x^2-\frac{(\Sigma x)^2}{N}\right]\cdot\frac{1}{N-1}$

Mean square between records of same cow $=R=\left[\Sigma x^2-\Sigma\frac{(\Sigma x)^2_c}{n_c}\right]\cdot\frac{1}{N-n_c}$

Mean square between cow means within herds $=R+\frac{N}{n_c}\cdot C=\left[\Sigma\frac{(\Sigma x)^2_c}{n_c}-\Sigma\frac{(\Sigma x)^2_h}{n_h}\right]\cdot\frac{1}{n_c-n_h}$

Mean square between herd means $=R+\frac{N}{n_c}\cdot C+\frac{N}{n_h}\cdot H=\left[\Sigma\frac{(\Sigma x)^2_h}{n_h}-\frac{(\Sigma x)^2}{N}\right]\cdot\frac{1}{n_h-1}$

Where: N=total number of records, n_c=number of cows, n_h=number of herds,
R=variance of records of the same cow,
C=variance of means of cows in the same herd, and
H=variance of herd means.

[4] The degrees of freedom between herds and between cows in the same herd are enclosed in parentheses to indicate that the variances (H and C) are not simply "mean squares" calculated by dividing the "sums of squares" by the appropriate degrees of freedom as is the case with R, the variance between records of the same cow.

[5] C is underestimated for raw production records, since a part of the variance between records of the same cow (i. e., that due to the age increase in yield) is represented only slightly in the variation between the means of different cows. A better estimate of C may be obtained by letting R=variance of the age-corrected record in C=(mean square between cows within herds$-R$)$\cdot\frac{n_c}{N}$. This gives repeatability values of 0.254, 0.252, 0.274, 0.270, and 0.258 for the 240-day, 305-day, 365-day, total lactation, and testing-year records, respectively.

BASIS OF CORRECTION FOR AGE

Age at calving accounted for about one-fifth (0.21) of the total variation in 240-day, but for progressively less of the variation in 305-day (0.17), 365-day (0.13), and total lactation production (0.10), owing to the much greater total variability of the longer records and to a slightly smaller variation between age classes (class intervals of 3 months) for the 365-day and total lactation.[6] Age at the beginning of the year accounted for only about one-twelfth (0.09) of the variation in testing-year yield, even though the total variation was about the same as for 305-day lactation yield (table 1). Plotting production on age (fig. 1) showed that the relationship was essentially linear up to about 5 years of age, and that no average increase in production occurred beyond that point. The only noticeable deviation from linearity was for the few first lactations (8 of a total of 274) of cows calving at less than 21 months of age.

Table 2 shows that the 240-day, 305-day, and 365-day lactation records starting under 5 years of age were significantly correlated with age only among records of the same cow (with the exception of the 240-day record between cows). The fact that persistency declines with age has been shown by Sanders (*25*, *30*), Turner (*35*), Gaines and Davidson (*13*), Gaines (*10*), Fohrman (*9*), and Schmidt (*32*). Sanders' conclusion (*30*) that the greatest age increase in production occurs within a few months after freshening is borne out here by the fact that no age increase in yield occurred beyond the eighth month of lactation (i. e., table 2 shows that the regression of 240-day lactation production on age was about the same as for 305-day and greater than for 365-day records).

Percentage correction factors for 240-day and 305-day lactation records were calculated from their respective linear regressions on age (up to 5 years) among records of the same cow, taking the mean 55-month yield as the mature level.[7] The regression of 305-day yield on age was fitted to the means of 365-day and total lactation yields (for ages under 60 months) in order to obtain the basis for calculating their age-correction factors. The 305-day regression was used for the longer records to exclude the influence of the significantly longer calving intervals and lactations (fig. 1) of first calvers from the age correction, since separate adjustment for variation in these influences was also to be studied.

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[6] For detailed analysis of variance tables, and for other tables pertaining to the data discussed in this paper, reference is made to the following: DICKERSON, G. E. EVALUATION OF SEVERAL ESTIMATES OF INHERENT PRODUCING ABILITY IN DAIRY CATTLE. Unpublished manuscript on file in the Univ. of Wis. library. The analysis of variance tables are summarized in table 9 of the present paper.

[7] For example, the 240-day fat yield values for each age, from which to calculate the percentage age correction factors, were obtained from the regression equation

$$Y=\bar{y}+(x-\bar{x})b$$

where

$\bar{y}$ = mean 240-day yield for ages under 60 months,

$\bar{x}$ = mean age at calving for ages under 60 months,

b = average increase in yield per month increase in age up to 60 months, among records of the same cow.

For 55 months, $Y=296.99+(55-40.26)3.23=344.6$

For 24 months, $Y=296.99+(24-40.26)3.23=244.5$

Correction factor for 240-day records of 24-month-old cows $=\frac{344.6}{244.5}=1.41$

TABLE 2.—*Analysis of covariance between age*[1] *and butterfat yield during lactation*

Source of variation	Degrees of freedom	First 240 days		First 305 days		First 365 days	
		r	*b*	*r*	*b*	*r*	*b*
			Pounds		*Pounds*		*Pounds*
Herd means	40	−0.036**	−0.641**	0.010**	0.217**	0.027**	0.626**
Cow means within herds	233	.196	2.489	.161*	2.656*	.117**	2.171**
Records of same cow	541	.667	3.230	.639	3.251	.562	3.053
Total	814	.506	3.103	.450	3.150	.389	2.952

[1] For lactations starting before 60 months of age. See also footnote 2, table 1.

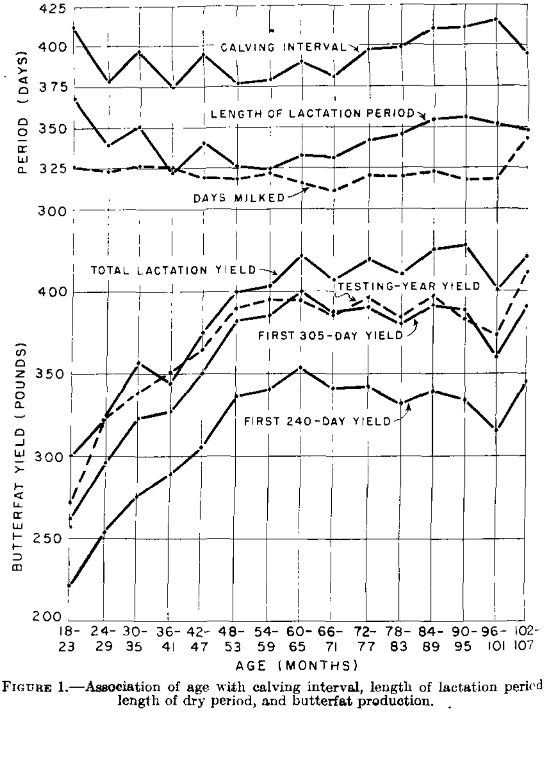

FIGURE 1.—Association of age with calving interval, length of lactation period, length of dry period, and butterfat production.

Factors for age correction of testing-year records were based on the mean testing-year yield and the regression of 305-day yield on age; their only important deviation for the 305-day regression was for the first year records which are lower relative to the first lactation records because the first interval between calvings was longer than later ones. Maximum testing-year production is reached earlier in terms of age at beginning of the testing-year than is maximum lactation yield in terms of age at calving. The age increase in yield occurs chiefly in the early part of the lactation period, and is therefore reflected in testing-year records beginning several months before the start of the lactation period in which the increase actually occurs.

RESULTS OF CORRECTION FOR AGE

Comparison of tables 1 and 3 shows that age correction increased the repeatability of all five kinds of records significantly (by one-sixth to one-half). The increase was greater for 240-day and 305-day than for the longer lactation records and the testing-year record. The increase was accomplished by a sharp reduction in the variation among records of the same cow, accompanied by an increase of similar proportions in the variation between cow means. The increased variation between cow means arose from the use of percentage correction factors greater than unity which add more to the average yield of high than to that of low producers. It has already been shown (7) that the increase in production with age is at least proportional to the producing ability of the cow and that, if anything, high producers are undercorrected and low producers overcorrected by percentage factors. The increase in repeatability of 365-day and total lactation records would have been somewhat greater had the age corrections applied been based on their own respective age changes instead of on the larger regression of the 305-day record on age, since the within-cow variation due to the age decline in calving interval and lactation length (fig. 1) would have been largely removed.

TABLE 3.—*Analysis of variance in age-corrected butterfat yield due to herd and cow differences* [1]

Source of variation	Variance symbols	Degrees of freedom	Variance in butterfat yield during lactation: First 240 days	First 305 days	First 365 days	Total lactation	Degrees of freedom	Variance in testing-year butterfat yield
			Pounds	*Pounds*	*Pounds*	*Pounds*		*Pounds*
Herds	H	(40)	815	1,150	1,211	1,164	(40)	1,517
Cows within herds	C	(233)	1,369	1,922	2,440	3,036	(233)	1,991
Records of same cow	R	1,298	2,683	3,663	4,804	6,895	1,182	4,490
Correlation between records in same herd	$\frac{H}{H+C+R}$	-------	.167	.171	.143	.105	-------	.190
Intraherd correlation between records of same cow	$\frac{C}{C+R}$	-------	.338	.344	.337	.306	-------	.307

[1] See footnotes 2, 3, and 4, table 1.

The smaller increase in repeatability for the testing-year record (change of 0.07 as compared to 0.11 for the 305-day record) was due to the fact that age-correction made only about one-third as great a percentage reduction in the variation among records of the same

cow for the testing-year as for 305-day lactation records, even though both the within- and the between-cow variation in unadjusted records were nearly identical for the two kinds of records. The portion of the variation in testing-year records associated with age at the beginning of the year (0.09) was smaller than that in 305-day records associated with age at calving (0.17). Since the age-increase in production occurs during the early part of the lactation period, it is naturally more closely associated with the age at calving than with the age at the start of a testing association's fiscal year, which bears less relationship to the time of freshening.

ADJUSTMENT OF LACTATION RECORDS FOR ENVIRONMENTAL VARIATION IN LENGTH

The influence of environmental variation in length of record can be minimized either by using a standard length of partial lactation short enough (e. g., 240 days) to avoid the influence of variation in length of time a calf was carried, as suggested by Gaines and Palfrey (*14*), Gowen (*15*), and others, or by adjusting longer kinds of records to a standard length basis as was done by Sanders (*27, 29, 30*). Presumably, the latter method would only be desirable if inherent differences in producing ability were more important in the later than in the earlier months of the lactation period. The repeatability in these data of 0.17 for dry period length (table 1) is an indication that important hereditary differences in persistency of lactation do exist, as maintained by Sanders (*25, 30*), Bonnier (*3*), and others; therefore it seemed worth while to compare the longer kinds of records adjusted to a mature age and standard interval between calving basis with the shorter partial lactation records corrected for age only.

DIRECT CORRECTION FOR CALVING INTERVAL

Variation in length of the interval between calvings was responsible for one-fourth (0.24) of the variation in total lactation records, and for one-tenth (0.11) of that in 365-day records, but for only a small amount (0.05) of the variation in production during the first 305 days of the lactation. Inspection of figure 2 and comparison of the squared gross correlation, 0.214 (table 4), with the between-calving interval classes portion of the variance, 0.236,[8] show that the association of total lactation yield with length of calving interval is essentially linear.

TABLE 4.—*Analysis of covariance of length of dry period and of lactation period, and total butterfat yield, with length of calving interval*[1]

Source of variation	Degrees of freedom	Length of dry period		Length of lactation period		Total butterfat yield during lactation	
		r	b	r	b	r	b
			Weeks		*Days*		*Pounds*
Between herds	40	0.437	0.0345	0.829	0.761	0.107**	0.184**
Between cows within herds	233	.254	.0191	.799	.787	.517	.906
Between records of same cow	1,173	.400	.0247	.909	.834	.521	.663
Total	1,446	.370	.0246	.878	.817	.463	.661

[1] See footnote 2, table 1.

[8] See footnote 6.

Percentage correction factors[9] were calculated from the average linear regression (0.66 pound per day increase in calving interval,

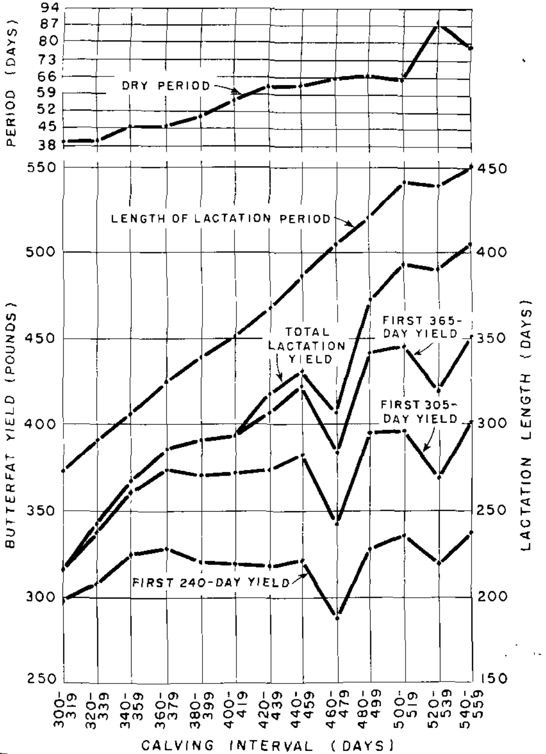

FIGURE 2.—Association of variation in length of calving interval with length of dry period, length of lactation period, and butterfat yield during lactation.

table 4) of total lactation yield on calving interval among records of the same cow. The modal length of 365 days was adopted as the standard length of calving interval to which correction was made. Extremely long intervals caused by breeding troubles accounted for a

[9] See footnote 6.

skewed distribution and a mean length of about 13 months (p. 56
similar to that found by Sanders (*27*), Gaines and Palfrey (*14*), a
Schmidt (*32*). Using figure 2 as a guide, the point at which t
first 305-day and 365-day lactation yield ceased to be associated wi
further increases in calving interval length was arbitrarily set at 3
and 460 days, respectively, in correcting these two kinds of "choppe
off" records to a 365-day calving interval basis. When the calvi
interval was more than 18 months, the first 365-day yield was co
rected and used as the estimated total lactation yield in a standa
365-day calving interval. Sanders' (*29*, *30*), observation that t
percentage factors for adjusting for length of service period (i. e
calving to conception) differed little between first and later lactation
or between persistent and nonpersistent cows, has been found t
extend also to different production levels within the same herd in the
data; hence a single set of factors for all records should prove fair
satisfactory.

A comparison of tables 3 and 5 shows that adjustment to a 365-da
calving interval basis increased significantly the repeatability of ag
corrected total lactation records and the between-herd portion of th
total variation in both the age-corrected 365-day and the total lact
tion records; other changes were not significant.[10]

The real gain in heritability resulting from adjustment for calvin
interval variation was greater than appeared from the increase
repeatability, since the influence of permanent cow differences i
calving interval length was included in the repeatability of the actu
but not of the corrected records.[11] There was probably a net gain i
the heritability of the age-corrected 365-day record with calving
interval adjustment for this reason.

It seems clear from the comparison of tables 3 and 5 that adjust
ment of the age-corrected 365-day and total lactation records for vari
tion in length of the interval between calvings only increases the
genetic worth to the same level as that of the 305-day record correcte
for age alone. However the results of several other methods of adjus
ing the age-corrected total lactation yield to a standard length basi
will be briefly reported here, lest it appear that the possibilities of th
longer kinds of lactation records were not exhausted.

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[10] The percentage reduction in the variation of age-corrected lactation records was considerably greate
particularly among records of the same cow, than the portion of the variation in unadjusted records origi
ally associated with calving interval length (table 4). Correction to a standard (365 days) 1 month belo
the average in itself would reduce the variation even if all records were reduced by the same percentag
Also the portion of the between-cow variation associated with calving interval had been reduced and th
within cows increased by the previous age correction.

[11] The significant repeatability of calving-interval length (0.07, table 1) may be due to intentional or i
voluntary delayed breeding of better producers, but it is doubtless also associated with the reproductiv
individuality demonstrated by Chapman and Casida (*6*), which may or may not be associated with produ
tive capacity. In adjusting all records to a 365-day calving-interval basis, the influence of permane
differences between cows in calving-interval length was removed. This was justifiable even if these di
ferences were largely hereditary, for otherwise the variation between cows in calving-interval length woul
tend to favor the lactation records of inherently "slow breeding" cows and penalize the more regular breede
ignoring the fact that the goal of selection is efficient reproduction as well as production. The significan
difference between the regression of 0.906 between cow means and that of 0.663 within cows in table 4 is ev
dence that the cows with the longer average calving intervals were higher producers for other reasons. The
cow differences associated with calving-interval variation are not removed by adjustment with factors base
on the within-cow regression (i.e., $\frac{(0.906-0.663)^2}{(0.906)^2}=0.07$ not removed). Had each cow been adjusted to he
own most probable "inherent" calving interval length as calculated by Lush's formula (*19*) (0.31 as muc
above or below the average for all cows as her own mean length for 6 records indicates), about 24 percen
of the between-cow variance associated with calving interval would have remained

$$(\text{i.e., } \frac{[0.906-0.663+(0.31\times 0.663)]^2}{(0.906)^2}=0.24)$$

instead of only 7 percent.

TABLE 5.—*Analysis of variance in age- and calving-interval-adjusted lactation records due to herd and cow differences* [1]

Source of variation	Variance symbols	Degrees of freedom	Variance in butterfat yield during lactation			
			First 305 days	First 365 days	Total lactation period	Per calving interval day
			Pounds	*Pounds*	*Pounds*	*Pounds*
Herds	H	(40)	1,218	1,312	1,367	0.01201
Cows within herds	C	(233)	1,835	2,079	2,116	.01429
Records of same cow	R	1,298	3,626	3,940	4,004	.03225
Correlation between records in same herd	$\frac{H}{H+C+R}$		.182	.179	.183	.205
Intraherd correlation between records of of same cow	$\frac{C}{C+R}$		.336	.345	.346	.307

[1] See footnotes 2, 3, and 4, table 1.

OTHER METHODS OF CORRECTION FOR CALVING INTERVAL

The age-corrected average yield per calving-interval day [12] was considered because of its close association with actual producing efficiency. However, its repeatability (table 5) was no greater than for the age-corrected total lactation record, since dividing by the calving interval greatly overcorrects for the influence of calving-interval variation among records of the same cow,[13] as is also indicated by the results of Chapman and Casida (*5*).

A method of adjusting the age-corrected total lactation record indirectly for calving-interval variation from the length of the lactation period was also tried. Direct adjustment of the age-corrected total lactation record to a standard lactation length with factors based on its average regression on lactation length among records of the same cow would not increase its repeatability because of the important inherent differences between cows in lactation length which are indicated by its repeatability of 0.12 (table 1) and its higher correlation with production between cow means (0.688) than among records of the same cow (0.594) as shown in table 6.[14]

[12] The average dry period for all previous records was added to the lactation length of last lactations for which the calving interval was unknown.

[13] The fact that variation in calving interval length was more highly correlated with the within-cow than with the between-cow variation in yield after correction for age means that the within-cow portion would have been reduced proportionally more by dividing by calving-interval length had the within- and between-cow regressions been the same (table 4). Since the between-cow regression was nearly as large as the mean age-corrected daily yield, while the within-cow regression was significantly smaller, dividing by the calving interval overcorrects for the within-cow influence of calving-interval variation but just removes cow differences in yield associated with calving interval (including the part represented by the difference between the within- and between-cow regressions, which is at least partly due to the better cows being bred later after calving).

[14] *Correction factors* based on the within-cow regression *would remove not only the environmental* part of the cow differences in production associated with lactation length (about two-thirds), but also the part due to permanent cow differences in lactation length (about another one-fourth), which are largely due to inherent differences in dry period length but also partly to individuality in calving-interval length. This would leave only about one-tenth, which is not the result of lactation length variation (i.e., represented by the between-cow regression being significantly greater than that within cows). That is.

$$\frac{([0.44\times0.81]+[0.56\times0.81]+[1.21-0.81])^2}{[1.21]^2}=1=$$

between-cow variance correlated with lactation length, when a cow's most probable inherent lactation length is 0.44 as much above or below the mean of all cows as her own average indicates (i. e.,

$$\frac{0.12\times5.75}{1-0.12+(0.12\times5.75)}=0.44,$$

where the intraherd correlation between lactation lengths of the same cow is 0.12 and the average number of records per cow is 5.75). Such adjustment of the age-corrected lactation yield should actually reduce the between- and within-cow variances in nearly the same proportions, since the age-correction decreased the proportion of the between-cow (from 43 to about 38 percent) and increased the proportion of the within-

TABLE 6.—*Analysis of covariance of length of dry period*[1] *and total butterfat yield during lactation period with length of lactation period*

Source of variation	Degrees of freedom	Length of dry period		Degrees of freedom	Total butterfat yield during lactation	
		r	*b*		*r*	*b*
			Days			*Pounds*
Herd means	40	−0.150**	−1.738**	40	0.350*	0.673*
Cow means within herds	233	−.266	−3.472	233	.688	1.211
Records of same cow	1,173	−.034**	−.504**	1,300	.594	.807
Total	1,446	−0.102	−1.432	1,574	0.585	0.885

[1] The regression of lactation length (days) on length of the following dry period (weeks) is given. See also footnote 2, table 1.

Inspection of figure 3 shows that variation in the length of lactations shorter than about 10 months was due largely to cow differences in dry period length, inasmuch as the length of calving interval remained nearly constant, and table 6 shows that dry period was only correlated with lactation length between the means of different cows. The method of correction for lactation length largely avoided removing the cow differences in lactation length under 10 months, since each record was corrected for the average calving interval length for the particular lactation length class with the same factors [15] used in correcting directly for calving interval variation.[16] Comparison of tables 7 and 5 with table 3 shows that this indirect method of calving interval adjustment reduced all portions of the variance in age-corrected total lactation records more than did direct adjustment for calving interval itself, because of the closer association of yield with lactation length (table 6) than with calving interval (table 4). However, the additional reduction was in the same proportion for all portions of the variance, leaving the repeatability and herd portions of the variance the same as for the direct method.

TABLE 7.—*Analysis of variance due to herd and cow differences for age- and lactation length-corrected total lactation records, age-corrected average daily yield between dry dates, and for testing-year records corrected for age and days milked*[1]

Source of variance	Variance symbols	Variance in butterfat yield during lactation			Variance in testing year	
		Degrees of freedom	Total lactation period	Average daily between dry dates[2]	Degrees of freedom	Butterfat yield
			Pounds	*Pounds*		*Pounds*
Herds	H	(40)	1,279	0.01153	(40)	1,339
Cows within herds	C	(233)	1,983	.01526	(233)	1,701
Records of same cow	R	1,298	3,656	.02610	1,182	3,941
Correlation between records in same herd	$\frac{H}{H+C+R}$		.173	.218		.102
Intraherd correlation between records of same cow	$\frac{C}{C+R}$		.352	.369		.301

[1] See footnotes 2, 3, and 4, table 1.
[2] Age corrected butterfat yield for total lactation period divided by the number of days in the total lactation period and the dry period preceding it.

[15] See footnote 6.
[16] For example, the average calving interval for lactations shorter than 280 days was 335 days, and th

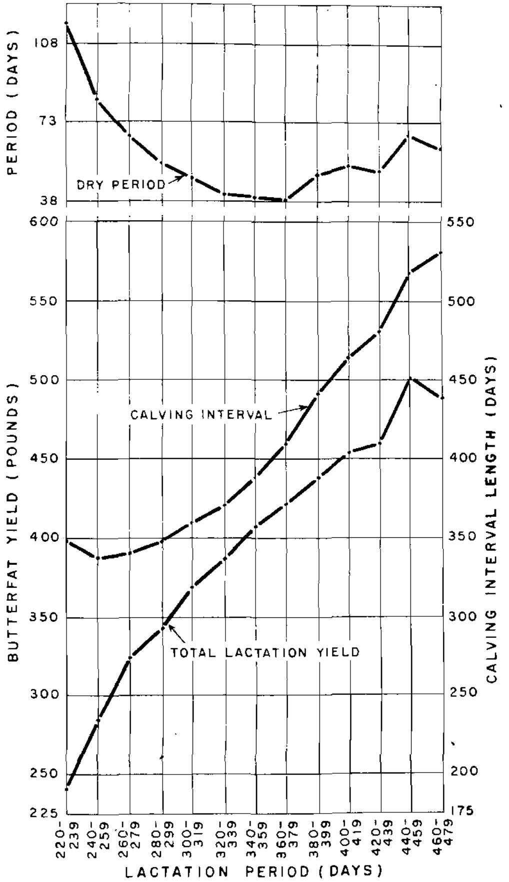

FIGURE 3.—The association of length of lactation period with length of dry period, length of calving interval and total butterfat yield during lactation.

ADJUSTMENT OF TESTING-YEAR RECORDS FOR NUMBER OF DAY MILKED

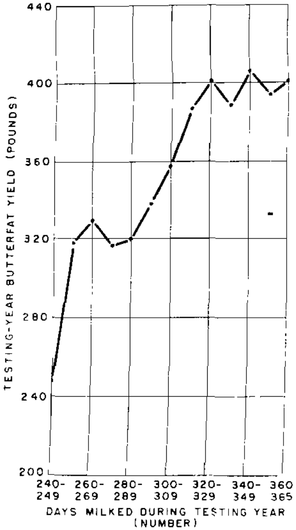

FIGURE 4.—The association of testing-year butterfat yield with the number of days milked during the year.

A comparison of tables ' and 3 shows that no gain i repeatability is obtained b adjusting the age-correcte testing-year record to a standard 325-days-milked basis wit percentage factors [17] based o the regression of productio on number of days milke among records of the sam cow, even though the associa tion was essentially linear, a shown in figure 4 and by . comparison of the square gross correlation (0.122, tabl 8) with the portion of th variance occurring betwee 10-day classes in days in mil (0.146). The explanation lie in the fact that variation i number of days milked ac counted for over twice a much of the variation in test ing-year records between co means as among records o the same cow (table 8), be cause of the significant repeat ability in number of day milked (0.08, table 1) and als because the number of day milked varies greatly in th part of the lactation period i represents in different testing year records of the same cow.[1]

TABLE 8.—*Analysis of covariance between days milked and testing-year yield* [1]

Source of variation	Degrees of freedom	r	b
			Pounds
Herd means	40	0.461	1.70
Cow means within herds	233	.431	1.44
Records of same cow	1,160	.291	.73
Total	1,433	0.350	1.01

[1] See footnote 2, table 1.

[17] See footnote 6.

[18] Since age-correction decreased the between-cow portion (from 0.18 to about 0.14) and increased th *within-cow portion (from 0.08 to about 0.09) of the variance in testing-year records associated with* day milked (tables 1, 3, and 8), correction for days milked with factors based on the within-cow regression woul presumably reduce the between-cow variance at least as much as that within cows (i. e.,

$$0.14-\frac{(1.44-74)^2}{(1.44)^2}\times 0.14 \geqq 0.09-\frac{(0.74-0.74)^2}{(74)^2}\times 0.09$$

leaving unchanged or slightly reducing the repeatability. Even though it were possible to remove only th temporary environmental differences between cows in days milked, the between-cow variance would b reduced about as much as that within cows. That is, $0.14-\frac{(1.44-0.74+(0.3\times 0.74))^2}{(1.44)^2}\times 0.14=0.08$ when a cows' most probable inherent number of days milked is only 0.3 (i. e., $\frac{0.08\times 5.3}{\ldots}$

OTHER INFLUENCES ON PRODUCTION

Of the remaining factors affecting butterfat production the feeding ractice was undoubtedly the major one, influencing herd differences ; production more than variation from year to year or from cow to ›w in the same herd, as Plum (*24*) has shown. Unfortunately, the formation on feeding practices in the present data is unsatisfactory ır the study of their influence on production.

ENGTH OF PRECEDING DRY PERIOD

In the present data ie influence of variaon in length of preding dry period was milar to that found y Sanders (*29*, *30*), uff (*34*), Arnold and ecker (*1*), and others, that production ppeared to increase ith length of dry ›riod up to about 1 onth and then deine for lactations folwing dry periods of ver 2 months (fig. 5). ctually, the apparntly lower production llowing the longer y periods indicates iat these are records the inherently non›rsistent, lower proıcing cows,[19] as also ›ted by Sanders (*30*). The rather abrupt crease in yield as dry ›riod is increased up › about 1 month corsponds to that found r persistent proıcers by Sanders *30*), and is interpreted › mean that a miniıum dry period of ›out a month is eeded in the physilogical preparation r maximum daily eld. Increasing the

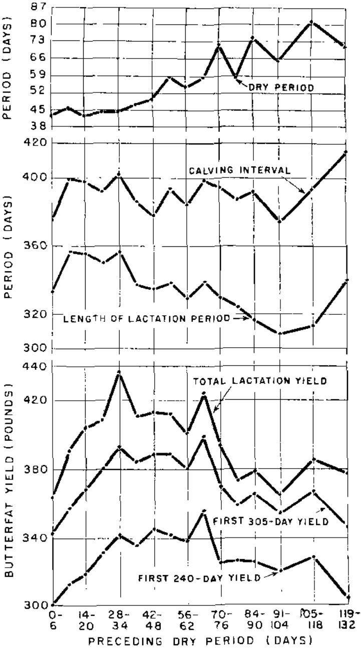

FIGURE 5.—Association of length of preceding dry period with length of calving interval, length of lactation period and butterfat yield during lactation.

[19] The increase in the following dry period length as the preceding dry period increased beyond 1 month 5) accounted for 0.09 of the following dry period variance, which corresponds to a gross correlation uding herd differences) of about 0.3 between successive dry periods of the same cow. This is only htly higher than the average gross correlation of 0.26 between any two dry periods of the same cow ulated from table 1. Since successive dry periods should be more alike than those separated by a greater difference, one concludes that the increase in dry period length with calving interval remaining con- t following longer preceding dry periods (fig. 5) was due to inherent cow differences in dry period length rsistency of production, and accounts for the apparent decline in production as the length of preceding

length of dry period beyond this point probably increases the persis ency, as shown by Sanders, but in figure 5 this tendency is obscure by the inherent cow differences in dry period length.

No correction factors for previous dry period variation are presente because: (1) The influence of dry period length on subsequent lacta tion yield was slight, even though significant (0.03 to 0.04 of tl variation in lactation records); (2) the association was greater in tl lower than in the higher producing herds (7) and undoubtedly differe for persistent and nonpersistent cows, and for second and late lactations, as shown by Sanders (*29, 30*); and (3) each cow's recor would need to be corrected to her own inherent length of dry perioc However, a rough method of correcting the total lactation recor simultaneously for variation in both preceding dry period and calvin interval length was attempted. The age-corrected total lactatio yield was divided by the number of days in the total lactation perio and the dry period preceding it.[20] By this method the lactation yiel is not overcorrected as much for calving interval variation as wa true in calculating the average daily yield for the calving interva since the longer calving intervals were accompanied by longer follow ing dry periods (table 4, fig. 2), but not by longer preceding dr periods (fig. 5). Furthermore, each cow's records tended to be cor rected to her own average dry period length, leaving inherent differ ences in persistency undiminished.

Comparison of tables 7 and 3 shows that both the repeatability an the between-herd portion of the variation in age-corrected tota lactation yield were significantly increased by dividing by the perio between dry dates, whereas no increase in repeatability resulte from dividing by the calving interval length (table 5). The variatio between cows was about the same as for the average age-correcte yield per calving interval day, for the lifetime average of these tw figures for a cow agree almost exactly. However, the variation be tween records of the same cow was significantly less for the age-cor rected daily yield between dry dates than for yield per calving interva day, giving the former the greater value in predicting real differences between cows in producing ability.

SEASON OF CALVING

Of the many investigators who have studied the influence of season of calving on production, Sanders (*26, 30*), Karl Schmidt (*32*), Fr. H. Schmidt (*31*), Marcq and Devuyst (*22*), Cannon (*4*), Plum (*24*), and others, all seem to agree that the unfavorable months for freshening are those associated with poorer feed conditions, particularly in the early months of the lactation period, and that the time of calving best suited to a particular locality depends on the seasonal variation in kind and quality of feeds available. In the present study season of calving (8 classes of 1½ months each) accounted for 0.036 of the variance in the 240-day yield for Holsteins, and for a progressively smaller proportion of the variance in 305-day and 365-day lactation records (0.019 and 0.010, respectively). The association with total

[20] Since the first lactation has no preceding dry period as such, the approximate modal dry period length (45 days) was added to the first lactation length to make its average daily yield figure more comparable to that of later records.

ctation records was not statistically significant (0.003).[21] Produc-
on was lowest for cows calving from April to September and highest r cows calving from October to March (fig. 6), the difference closely sembling that found · Plum and by Can-n in Iowa testing sociation records. The smaller relative fluence on the longer nds of lactation cords was due to the eater total variance r longer records, and the barely signifi-nt tendency shown figure 6 and table 9 r cows freshening om April to September to have the nger lactations and lving intervals. Because of its relative unimportance and e tendency, shown Sanders (*30*), for e records of persistent cows to be less fected than those of npersistent cows, no rrection for season calving was attempted.

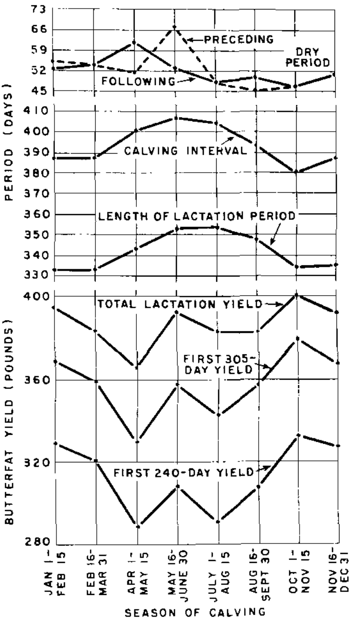

FIGURE 6.—Association of the season of calving with calving interval, length of lactation period, length of preceding and following dry period, and butterfat yield during lactation.

DISCUSSION OF RESULTS

The central objective of this investigation was to determine hat kind of modified roduction record ost accurately reects hereditary differences in producing bility. The data udied did not permit direct determination of the portion of the variation in production which was hereditary in the narrowest sense (i. e., transmitted om parent to offspring). Therefore, it was necessary to use a closely lated criterion, the portion of the variance in yield associated with herent (i. e., permanent or average lifetime) differences between ows in the same herd environment. As Lush and his coworkers

[21] See footnote 6.

(*20, 21*) point out, inherent differences between cows include hereditary differences which are transmitted, those which may not be transmitted (such as effects of dominance and epistasis), and permanent differences in environment (i. e., in addition to those which would occur by chance alone).

TABLE 9.—*Percentage of variance in several environmental factors and in different kinds of production records associated with various environmental influences* [1]

Variables affected	Number of records	Percentage of variance for indicated environmental influences					
		Total variance [2]	Age	Preceding dry period	Season of calving	Calving interval length	Lactation length
Calving interval	1,447	5,460	1.5	0.6**	0.7*	--------	------
Lactation period length	1,574	4,902	1.4*	2.2	.6*	78.4	-------
Dry period length	1,447	24.1	1.6	8.9	.7*	18.4	-------
First 240-day butterfat yield	1,574	5,396	21.2	3.8	3.6	1.1**	3.9
First 305-day butterfat yield	1,574	7,394	16.9	3.9	1.9	4.8	11.9
First 365-day butterfat yield	1,574	8,971	13.4	3.9	1.0	11.1	21.7
Total lactation period butterfat yield	1,574	11,214	10.5	3.3	.3**	23.6	35.7
Days milked, during testing year	1,434	878	1.4	--------	--------	-------	-------
Testing-year butterfat yield	1,434	7,332	8.7	------	--------	--------	[3] 14.6

[1] See footnote 2, table 1.
[2] First lactations were necessarily omitted in studying the influence of preceding dry period length, so that the total variances for variables associated with it differed slightly from those given in this table. The same applies to variables associated with calving interval length which was unknown for the last lactations of some cows.
[3] Days milked.

For the purpose of determining the relative genetic worth of different kinds of production records, the fraction of the within-herd variance associated with inherent or permanent differences between cows (i. e., the average within-herd correlation between records of the same cow, or "repeatability") is nearly as useful as the fraction of the within-herd variance due to hereditary differences transmitted from parent to offspring would have been, since these two fractions bear a fairly constant relationship to each other. The quantity included in the numerator of the first fraction but not in the numerator of the second (the dominance and epistatic variations not transmitted, and permanent effects of environment) would be the same for different measures of producing ability, unless some discount the relatively small permanent effects of environment more than others. Lush and Arnold (*20*) have estimated that the second fraction (heritability) comprises about two-thirds of the first (repeatability) for butterfat production records.

An instance of removal of permanent differences between cows occurred when the influence of the 6 percent of within-herd variation in calving interval due to cow differences was removed by correction to a yearly calving basis. The net gain in heritability from such correction therefore was greater than indicated by the increase in repeatability.

It has been shown that the effect of an environmental influence on production among records of the same cow may not be represented truly in the gross association because of (1) the effects of selection on the data (e. g., as in culling with age), (2) hereditary differences in an influence which is also an environmental source of variation among

ınd (3) permanent environmental differences between herds or beween cows in the same herd (e. g., as in length of calving interval). n the present study these factors have been taken into account and he average association among records of the same cow determined ›y the covariance technique whenever the association was a linear one.

Adjustment with percentage correction factors assumes that cows ıf different levels of persistency or of producing ability make about he same proportional (but not absolute) change in production with ı given environmental difference. Sanders (*26, 28, 29, 30*) has demontrated that persistent and nonpersistent cows do not make the same ›ercentage change with age, previous dry period, or season of freshnıng, as previously discussed, and that the effect of dry period differs or second and later calvers. In the present data, the age increase t different levels of producing ability did not differ significantly from hat assumed in using percentage age-correction factors, but the ncrease in production with longer preceding dry periods was greater ather than less for cows in the lower producing herds, making the use ıf a single set of factors for dry period length on all records entirely ınsatisfactory (*7*). Percentage correction factors for calving interval ·ariation should be fairly satisfactory, however, judging from Sandrs' (*27*) (*30*) finding that the percentage change in total lactation ·ield with service period (calving to conception) differed little for ıersistent and nonpersistent producers or for first and later lactations, nd from the fact that no consistent differences in the percentage facors were found between different production levels of herds or of ·ows within herds in the present data.

Sanders (*30*) and Gaines (*11*) have studied the increase in repeatbility of production records with correction for environmental inluences. Sanders found that correction of the total lactation milk ·ield for season, age, dry period, and service period increased the ‹ithin-herd correlation between records of the same cow from 0.52 o 0.73. In the present study, correction of the total lactation yield or age and calving interval (no correction for dry period and season) ncreased the repeatability from 0.26 to 0.35. Sanders' corrections or dry period and season would have increased the between-cow and urther reduced the within-cow variance. Overlooking the differnces in level of repeatability in the two studies (discussed later), the ›roportional gain in repeatability with correction for environmental nfluences is similar in both. The failure of correction for age and ıumber of daily milkings to increase the repeatability of Holstein Ierd Improvement Register 365-day lactation records reported by Gaines was probably due to the important cow differences in number ıf daily milkings in his data removed by such corrections. Cow lifferences remaining after the corrections would be more largely ıereditary than those in the actual records; thus the heritability, or ·alue of the records for selection purposes, was undoubtedly increased ›y the adjustment for age and daily milkings.

Which of the five kinds of production records best measures inherent lifferences in producing ability? It is apparent that the repeatability ıf age-corrected 240-day and 305-day records was just as great as or the 365-day and total lactation yields corrected for both age nd length of calving interval (table 10). The age-corrected testing-·ear record was lower in repeatability than were the age-corrected

240-day, 305-day, and 365-day lactation records, because the association between age at calving and lactation yield was much closer than between age at the beginning of the year and testing-year yield (table 9) for reasons previously discussed (p. 568). The difference which Harris, Lush, and Shultz (*18*) found in favor of the age-corrected 365-day lactation record (0.34 to 0.31) was duplicated in the present study (0.34 to 0.31). Furthermore, the lactation record may be susceptible of further improvement in accuracy by proper correction for length of preceding dry period and for season of calving, while the testing year is not. However, the testing-year record, in common with the average daily yield for the calving interval or for the period between dry dates, does have the possible advantage of reflecting cow differences in regularity of reproduction which the lactation records do not. Thus it appears that the age-corrected 305-day lactation record now in general use is fully as satisfactory for the purposes of selecting for producing ability as any of the other kinds of records. It also becomes available sooner and entails less work in computation than the age- and calving-interval-corrected 365-day or total lactation records.

TABLE 10.—*Summary of relative genetic worth of various estimates of producing ability and of the importance of herd and cow differences in factors influencing yield*

Kind of record	Correction	Variance between records of same cow, R [1]	Correlation between records in same herd, $\frac{H}{H+C+R}$ [1]	Intraherd correlation between records of the same cow, $\frac{C}{C+R}$ [1]
ESTIMATES OF PRODUCING ABILITY				
First 240-day butterfat yield	None [3]	3,612	0.14	[2] 0.23
	Age	2,683	.17	.34
First 305-day butterfat yield	None [3]	4,820	.16	[2] .23
	Age	3,663	.17	.34
	Age, calving interval	3,626	.18	.34
First 365-day butterfat yield	None [3]	5,735	.14	[2] .26
	Age	4,804	.14	.34
	Age, calving interval	3,940	.18	.35
Total lactation period, butterfat yield	None [3]	7,513	.09	[2] .26
	Age	6,895	.10	.31
	Age, calving interval	4,004	.18	.35
	Age, lactation length	3,656	.17	.35
Age-corrected average butterfat yield per calving interval day.		.03225	.20	.31
Age-corrected average butterfat yield per day between dry dates.		.02810	.22	.37
Testing-year, butterfat yield	None [3]	4,886	.19	[2] .24
	Age	4,490	.19	.31
	Age, days milked	3,941	.19	.30
FACTORS AFFECTING PRODUCTION				
alving interval ... days		4,718	.07	.07
ry period length ... weeks		17.90	.10	.17
actation period length ... days		4,077	.05	.12
eriod milked during testing year ... do		926.4	.09	.08

[1] See footnote 3, table 1.
[2] Underestimated by about 0.02. See footnote 5, table 1.
[3] Except for number of milkings per day to a two-time basis.

Since the age-corrected 240-day yield is no less repeatable than the 305-day or 365-day yields in these data, one wonders whether a still shorter partial lactation might not do as well. Gaines (*12*) has shown that the average correlation between two successive actual fat records of the same cow in Guernsey Advanced Register records from many herds increases steadily from 0.51 for the first 2-month partial lactation to 0.68 for 365-day lactation yield. However, even though the first 2-month yield was just as repeatable as the 10-month yield, the latter would be preferable because it is more highly correlated with the goal of selection—high average lifetime production. The first 2-month yield measures only the maximum productive capacity; the 10-month yield measures the degree of persistency as well as maximum producing ability.

Now the question arises, what is the actual repeatability or predictive value of the best estimate of producing ability within the individual breeder's herd? In comparing the estimates of the within-herd repeatability found in various studies the effect of the number of records per cow on the estimate of repeatability must be considered. One would expect the environmental variation between successive records of a cow to be less than between records separated by a number of years during which greater changes in age, management conditions, disease, etc., may influence her production, as shown by Berry and Lush (*2*). Also, Seath's results (*33*) indicate that inherent differences between cows are likely to be smaller in a population of cows from which those culled before completing their fifth lactation have been eliminated than in a population which includes all cows with two or more records. Furthermore, time trends and yearly variations in management conditions are more likely to cause "permanent" environmental differences in production between cows having only 2 records each than among cows having 5 to 10 records each.

In Gowen's (*16*) Holstein Advanced Register data, 0.33 of the variation was due to herd differences. Thus his correlation between two successive records of the same cow would become 0.51 when calculated on a within-herd basis, as in the present study. Assuming the same herd variation, his repeatability figure for Guernsey (*16*) milk yield would become 0.55 and for Jersey (*17*) milk yield between 0.4 and 0.6, both of which are similar to his estimate of 0.54 from a single Jersey herd (*15*).

In studies of Harris, Lush, and Shultz (*18*) and Plum (*24*) all cows with two or more records were used, herds tested for 3 or more years being included. Gowen's various estimates of the within-herd repeatability of 0.5 or thereabouts were higher than Plum's estimate of 0.4 because only successive records of a cow were used, and perhaps also because of greater differences in environment affecting individual cows in Advanced Register testing (in number of daily milkings, especially). Sanders' estimate (*30*) of 0.7 is still higher than Gowen's, because in addition to using only two successive records of a cow he adjusted for variation in dry period and season as well as for age and calving interval differences. The lower repeatability of age- and calving interval-corrected lactation yield in the present study (0.35) is apparently due to the more select group of cows taken from each herd (only those with five or more records) and to smaller permanent

environmental differences between cow means (three and four daily milking records were corrected to a two-milking basis here, while Plum's were not), since the between-cow variance in age-corrected 240-day yield was smaller (1,369) than in Plum's data (1,707), while the within-cow variances were similar (2,683 as compared to 2,554). Gaines and Palfrey's estimate (*14*) (0.5 for cows with 10+ lactations) includes herd differences and would probably be around 0.3 on a within-herd basis, being about as much lower than Plum's as in the present study and for similar reasons. Permanent environmental differences between cows (from year-to-year variation in herd environment) probably account for less of the repeatability in Gaines and Palfrey's and in the present study than in any of the others, including Sanders'. The repeatability of 0.33 obtained by Harris, Lush, and Shultz for age-corrected 365-day lactation fat yield is lower than Plum's estimate of 0.40, probably because Plum used age-correction factors carefully calculated from his own data, while the former used the old 70-, 80-, and 90-percent factors for 2-, 3-, and 4-year-old records. Plum's estimate of 0.4 is the one which is most nearly typical of the average within-herd repeatability of age-corrected 240-day or 305-day lactation records in breeders, herds where all cows are production-tested every year, and some are milked three times daily. Adjustment for frequency of milking would lower this repeatability figure slightly.

No satisfactory method has been devised for determining what part of the differences between herd averages is due to hereditary differences between the cows. Plum (*24*) has shown that herd differences in feeding practice are associated with at least one-third of herd differences in age-corrected 240-day lactation yield, and has estimated that all environmental herd differences probably account for over half of the herd variation in yield, leaving somewhere near two-fifths to be explained by hereditary differences (including effects of dominance and epistasis) between the cows in different herds. Harris, Lush, and Shultz (*18*) are in agreement with Plum in his conclusion that herd differences account for about one-third of the total variation in age-corrected yield. In the present study, only one-fifth to one-sixth of the total variation in age- and length-corrected lactation butterfat yield was accounted for by differences between herd averages (table 10). A comparison of the analysis of variance in age-corrected 240-day lactation yield for Plum's and the present study reveals that the variance between herds is less than half as great in the present study (815 as compared to 2,199), while the variance among records of the same cow is quite similar (2,683 as compared to 2,553) in the two studies.

Two facts account for the smaller differences between herds in this study. (1) Selecting only herds tested for at least 5 consecutive years automatically eliminated many of the lower-producing herds. The average production in the herds used was at least 50 pounds of fat above the mean for *all* tested herds. This in itself reduced the variation between herd means in these data below that found in the data of Harris, Lush, and Shultz, and Plum, which included herds tested for only 3 years. (2) Records during which a cow was milked three or four times daily were adjusted to a twice-a-day milking basis in this study but not in the other two studies. Since there was considerable uniformity in the number of daily milkings ... within each

herd, correction to a two-daily-milking basis reduced the herd differences due to management practices. The extent to which the smaller herd variation in this study was more largely hereditary in origin than that found by Harris, Lush, and Shultz, and by Plum, depends on the degree to which environmental differences between herds were minimized more than was hereditary variation by using only herds which had been tested for a long time and by correcting all records to a two-daily-milking basis.

To the skillful breeder, production records are considerably more useful in comparing animals than these studies would indicate, since he knows and can make more accurate allowance for the influence of changes in age, feeding, and other environmental fluctuations than can possibly be incorporated in corrections applied to all herds alike. This means that the careful breeder will make fewer mistakes in his selections of breeding stock within his own herd or in herds with which he has constant contact than in selecting animals from outside herds. As Berry and Lush (*2*) point out, the large amount of unexplained variation between records of the same cow means that the lifetime average production will always be more useful than single records in comparing the inherent producing ability of different cows, and that the herd average and the number of records each cow has should be considered in comparing animals.

SUMMARY

Lifetime butterfat production records of 274 Holsteins from 41 herds were studied to determine what adjustments for environmental influences are advisable and the relative usefulness of five kinds of adjusted records (240-day, 305-day, 365-day total lactation, and testing-year) in selecting for producing ability. The average within-herd correlation between records of the same cow (repeatability) was the criterion used in evaluating adjustments and comparing kinds of records, since it measures the degree of accuracy with which records of different cows indicate real differences in their relative producing ability. Correction factors for influences linearly associated with production were based on the average association among records of the same cow determined by the covariance technique. The analysis of variance was used to determine the relative importance of each environmental influence as a source of variation in production and in other environmental influences (summarized in table 9), and the repeatability and between-herd portion of the variation in the various kinds of actual and adjusted records and factors influencing production (summarized in table 10).

A significant increase in repeatability of all five kinds of records resulted from age-correction with percentage factors based on the essentially linear association of production with ages up to 5 years. The increase was greater for 240-day or 305-day lactation (one-third) than for testing-year records (one-fifth), chiefly because age at the beginning of the testing year was less closely associated with the age change in production (which occurs during the early part of the lactation period) than was age at calving. The increase was smaller for 365-day and total lactation records (one-fifth and one-ninth) because of the greater variation from other sources in the longer kinds of lactation records.

Correction for calving interval to a 365-day basis, with factors based on its linear association with production, increased the repeatability of total lactation (and probably the heritability of 365-day) records significantly, in spite of removing permanent (hereditary and environmental) cow differences in calving interval length. The same increase in repeatability was secured by an indirect method of correcting for calving interval from the lactation length of each record. Dividing the age-corrected total lactation record by the number of days between calvings greatly overcorrected for the influence of calving interval among records of the same cow and did not increase the repeatability; however, dividing by the number of days in the lactation period and preceding dry period tended to adjust each cow's records to her own average preceding dry period length and more satisfactorily corrected for variation in the interval between calvings, increasing the repeatability significantly.

There were important differences between cows in the same herd in inherent dry period length (which reflects persistency), and hence in lactation length and days milked during the testing year, which made adjustment of production records for only the environmental variation in these influences difficult or impractical. The cows with the shorter mean lactation lengths were much lower producers and had much longer dry periods than would have been expected from the average association among records of the same cow. The same was true of the cows milked fewer days during the testing year on the average. Lactation length could be used only indirectly to predict the calving-interval length for which adjustment was made. Adjustment of the age-corrected testing-year record for days milked with factors based on the linear within-cow regression did not change the repeatability. No percentage factors for adjusting for preceding dry period length were used, since its influence varied inversely with the herd production level.

Season of calving was a relatively unimportant source of variation in production and no correction factors are presented. The effects of feeding were not studied. The values found for the between-herd portion of the variance (one-fifth to one-sixth) and the repeatability (one-third) of age and length-corrected records are lower than would be found among records of all cows in production-tested herds because only cows with records for at least their first five lactations from herds with three or more such cows were selected for this study.

The age-corrected 240-day and 305-day lactation records were equal to the age- and calving-interval-corrected 365-day and total lactation records and superior to the age-corrected testing-year record in repeatability; the portion of the variance due to herd differences was identical for these five kinds of records; and the average age-corrected daily yield between dry dates was at least equal to any of these five estimates of producing ability in both respects. The age-corrected 305-day record is probably the most satisfactory for selection purposes, since it becomes available sooner and is easier to compute than any of the other kinds of records except the age-corrected 240-day record, which is less desirable than the 305-day record because it is not as closely correlated with average lifetime production—the ideal measure of performance.

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EARLY RECOGNITION OF THE FREEMARTIN CONDITION IN HEIFERS TWINBORN WITH BULLS [1]

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INTRODUCTION

A freemartin, according to definition by Webster, is a sexually imperfect, usually sterile, female calf, twinborn with a male. Dairy-cattle-breed associations refuse to register a female twinborn with a bull until after she has freshened. The possibility that such individuals will be capable of reproduction is so slight that many breeders destroy them soon after birth. However, breeders of high-producing herds, in which heifer calves are valuable, are often willing to spend the time and expense to raise such individuals to breeding age in the hope that they will prove to be sexually normal.

A number of cases of twin births involving both sexes have occurred in the dairy herd of the Bureau of Dairy Industry at Beltsville, Md. The results of studies of the females of these mixed twins are presented herein to provide additional knowledge about the nature of the abnormalities found in freemartins, with the hope of furnishing criteria that will aid in determining at an early age, whether the female of mixed twins is a freemartin or is normal.

REVIEW OF LITERATURE

The freemartin has been known to cattle breeders since before the establishment of the Roman Empire. The sterile cow born twin with a bull was referred to by Varro, a writer who died in 28 B. C. It was called "taura," which apparently meant "barren cow." Although the condition has been recognized for some 2,000 years the origin of the term "freemartin" is obscure. According to one authority the word "free" meant "willing" or "ready to go," as the freemartin was supposed to be an especially willing worker. It has been proposed also that the word "free" was used to signify exemption from reproduction (sterile). Another authority saw in the term a contraction of the words "ferry," "ferow," or "farrow," which appear to be associated with the Flemish "varvekoe"—a cow that gives no milk—and with the West Flemish "varwekoe"—a cow that has ceased to be capable of producing offspring. It is not difficult to imagine an association between the two words "free" and "farrow."

There is probably greater speculation about the word "martin." It may have been derived from the Irish and Gaelic "mart" meaning heifer or cow. Efforts have been made to trace it to St. Martin who, according to legend, once cast the devil from a cow. Moreover St. Martin is said to have been the patron saint of twins and unusual

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fecundity. Another explanation offered is that on or near November 11, which was called Martinmas day in Scotland and England, it was customary to slaughter cattle the meat of which was salted for winter use and called martinmas-beef. An early English dictionary referred to martin as "not a true heifer, but an undeveloped male with many of the characteristics of the ox, and generally fattened and killed about Martinmas." It has been suggested further that the freemartin may have been given that designation because its meat was so choice that it was reserved for St. Martin's—a great feast day. Moreover the words "mart," "maert," "mert," and "mairt" appear to have been used in Scotland and parts of England in referring to the cow or ox fattened for slaughter and salted or smoked for winter use. Hart (*32*)[3] showed that it is not difficult in view of these facts, to imagine such an individual being referred to as the "farrow-mart-one," or in Scotland as the "farrow-mart-yin," either of which might have been corrupted or shortened into "freemartin."

Despite the general knowledge of the existence of the freemartin condition in cattle and the various notions concerning it that had prevailed through the centuries, Hunter (*37*) appears to have been the first (1779) to place detailed anatomical descriptions of the freemartin on record, and many of the articles published on the subject during the last 150 years have made specific reference to John Hunter's freemartin as a basis for comparison. Most of the early publications were limited chiefly to descriptions of its appearance, sexual behavior, and morphology. In recent years, however, its occurrence has ıttracted the interest of biologists, embryologists, and geneticists.

Most writers on the subject have held to the theory that the 'reemartin, which resembles the female more than the male externally, s a modified female. Some have appeared to be uncertain as to its ;enetic origin, and others have contended that it is a modified male. t is not surprising that opinions should differ on this point because ome freemartins having the external appearance of females have ɔeen found to be almost devoid of internal genitalia, whereas others ɪf similar external appearance have been found on dissection to possess ·ssentially all of the male genitalia in modified form.

Another question having an important bearing on the matter of ;enetic origin is whether the individuals in multiple births involving reemartins develop from the same ovum or from separate ova. If ·oth originate from 1 ovum, the freemartin presumably must be a ıodified male as identical twins are always of the same sex. Hart *33*) and Cole (*6*) contended that the freemartin and its twin result 'om a division of the male zygote. Gowen (*22*, *23*) on the other and, concluded that identical twins do not occur in cattle. However, isslowský (*42*), Kronacher and Sanders (*45*), Kronacher and Hogreve *44*), Kronacher (*43*), Lush (*52*, *53*), and Jewell (*39*) have offered ıirly convincing evidence that identical twins may occur in cattle. illie (*47*), in his studies of twin embryos in a packing house, proved most beyond question that when a single fetus occurs only 1 corpus teum is present and that almost invariably when twin fetuses occur corpora lutea are present as evidence of the liberation of 2 ova—1 om each ovary—regardless of the sex of the fetuses. Lillie (*50*) ıowed that in 127 cases of twinning where the maternal ovaries

[3] Italic numbers in parentheses refer to Literature Cited, p. 620.

were available for examination, 126 showed 2 corpora lutea, and that in every case involving two-sexed twins 2 ova had been concerned. Most of those who claim the existence of identical twins in cattle admit their rarity. Evidently monozygotic twinning in cattle is the exception rather than tbe rule.

EXPLANATION OF FREEMARTINISM

Lillie (*46, 47, 48, 49, 50, 51*), who examined a large number of twin fetuses in a packing house, offered a theory explaining the occurrence of the freemartin in cattle which has been rather widely accepted. More than 96 percent of the bovine twins he examined were monochorial (inclosed in a common placenta); the two fetuses usually had developed in such a way that the blood vessels of the two circulations were joined and a constant interchange of blood had taken place between the two developing fetuses. If both fetuses were male or both were female neither was detrimentally affected by this common circulation, but if one was male and the other female a sterilization of the female took place. In some such cases the development of the female reproductive organs was suppressed, and in extreme cases certain male organs developed in the twin that was originally female. He explained the occasional occurrence of fertility in the female of mixed twins on the theory that, even though monochorial, the vascular systems of the two fetuses do not necessarily become joined.

Although Lillie is usually credited with having performed the original research work leading to the conclusions outlined, he (*49*) gave credit to Tandler and Keller (*63*) for having anticipated certain of his observations. Tandler and Keller found that twin cattle fetuses were inclosed in a common chorion and usually had a joint circulatory system resulting in typical freemartin genital organs in the female if the twins were originally of opposite sex. Nevertheless, for purposes of brevity the foregoing explanation of the cause of the freemartin will be referred to in the following discussion as Lillie's theory.

The histological studies of Lillie's specimens by Chapin (*4*) showed that the interstitial cells of the testis, the secretion of which supposedly determines the development of secondary sexual characters, are produced earlier in fetal life than the corresponding cells of the ovary. Thus, in the case of vascular fusion in mixed twins the sex hormones of the male fetus pass into the circulation of tbe female embryo early enough to interfere with the development of the mechanism controlling female secondary sexual characters, and the sex organs of the female, which are in an indifferent stage, develop toward the male condition. Chapin attributed the great variation in the degree of alteration or reversal in the reproductory organs of the freemartin to differences in the stage at which the interstitial secretions of the male are introduced into the female embryo, and to the amounts introduced.

Buyse (*3*) attributed the extreme modification he found in a 2½-year-old Brown Swiss freemartin to the fact that it was one of triplets of which the other two were males. However, Pearl (*55*) showed that one male of triplets was capable of sterilizing two females. Similar results were shown by Bissonnette (*2*). Hutt (*38*) cited a case of bovine quadruplets in which one male apparently modified three females and caused them to become freemartins. Lillie (*51*) and

Bissonnette (*2*) concluded that the amount of hormone does not determine the degree of sex modification but that it is an "all-or-none" reaction as far as freemartin formation is concerned. The extremely small quantity of the hormone required to transform the female into a freemartin is particularly noteworthy. Lillie (*51*) showed that in one case a male fetus weighing less than 4 gm. had produced male hormones sufficient to bring about a practical inversion of the ovary in the female twin.

NUMBER OF FREEMARTINS STUDIED AND METHODS EMPLOYED

Reports of the occurrence of the freemartin condition in cattle are too numerous to discuss in detail. Much of the work has been carried on with embryos obtained at slaughterhouses. Other studies have been based on living animals ranging up to several years of age. Many of the reports on record have been based on the study of a small number of individuals. Most of the cases were associated with twins but at least 2 reports involved triplets, and 1 report described freemartinism in connection with the birth of quadruplets. Magnusson (*54*) examined 66 freemartins, most of which were 2 to 3 years of age, and described 11 of them in detail. Lillie (*51*) gave some idea of the large number observed in crediting Keller and Tandler with 91, Lillie with 39, Numan with 8, and Luer with 113. These studies have shown a very marked variation in degree of sex modification.

FREEMARTINISM IN OTHER SPECIES OF ANIMALS

Sex intergrades are known to occur in many species of mammals. Numerous cases of intersexuality closely simulating freemartinism have been found in the goat. Davies (*17*) described a number of cases of what he called caprine freemartins. These animals generally have a tendency to be female (sometimes even being effeminate looking early in life) but become strongly masculine in appearance toward the end of the second year. According to Davies the abnormality is believed to occur in at least 2 percent of goats and appears more frequently in some breeds than in others. He stated that apparently 2 intersexual kids never occur at the same birth, and indicated that in goats the condition may be found (1) in single births, (2) as twin to a normal male, (3) as twin to a normal female, (4) as 1 of triplets with normal male and female, and (5) as 1 of triplets with 2 normal males. One case examined in detail by Davies was described as "exactly similar to the condition described by John Hunter in freemartins in cattle." Rickards and Jones (*58*) also described twin goats that appeared to be typical females at birth, in which the anatomical structure later became strongly masculine although the mammary development appeared to be normal for the female. A study of 25 similar cases in goats was reported by Crew (7), whose description was nearly the same as that of Davies and of Rickards and Jones.

Apparently the occurrence of sex intergrades is less frequent in sheep than in cattle or goats. Strebel (*61*) gave figures which indicate that sterility is almost as likely to occur in the ewes of like twins as in ewes twinned with males, and by no means as likely to occur in sheep as in cattle when the twins are of opposite sex. Fraser-Roberts and Greenwood (*19*) reported in detail on a ewe lamb with testicles which

they considered to be similar to the freemartin. Hunter (*37*) referred to the frequency of sex intergrades in sheep and described them in some detail. Numan, according to Hart (*32*) described intersexuals in both goats and sheep. Lillie (*50*) referred to the frequency of chorionic fusion and the infrequency of vascular fusion. Fincher and Williams (*18*) stated that the chorionic fusion without vascular fusion is the rule in both sheep and goats, and that sometimes vascular anastomosis occurs and occasional asexual young are recorded. Williams (*64*) referred to the citation of one case of limited vascular anastomosis in the ewe. Crew (*7*) reported a case which he stated was very similar to 34 other cases in the goat, the pig, the horse, and in cattle. Gurlt, according to Hart (*32*), concluded that intersexuals in both goats and sheep are analogous to the freemartin.

Hunter (*37*) indicated that intersexuality in the horse is very frequent. He described one case in which the testicles had descended and appeared like an udder. According to Hart (*32*), equine hermaphroditism was reported by Gohier, and by Wotton in 1841, and also by Sebald. Crew (*7*) mentioned two cases of intersexuality in the horse very similar to the intersexual individuals found in the goat, the pig, cattle, and sheep.

Sex intergrades appear to be fairly common in swine. Kingsbury (*41*) described in detail a case in a pig 9 months of age. Lillie (*50*) stated that in swine the fetal membranes often fuse but that vascular anastomosis occurs only exceedingly rarely. Hughes (*36*), however, reported extensive studies of 400 pig uteri of which 4 showed fusion of fetal membranes and vascular intercommunication with abnormalities in the sex equipment of one or the other component twin. Three of the cases were heterosexual. In the fourth both were males. She stated that females of the heterosexual pairs would undoubtedly have developed into typical sterile freemartins such as occur in cattle. Cohrs (*5*) observed a number of cases of different types of embryonic fusion and different degrees of vascular anastomosis. Fincher and Williams (*18*) described a six-fetus chorion in which 4 were males and 2 were perfect females. Abundant vascular anastomoses between the 6 fetuses of mixed sex had failed completely to injure or delay the physiological development of the reproductive system of the females. Crew (*7*) indicated a close similarity between the intersexuals of swine and those of horses, cattle, sheep, and goats, but later (*8, 10*) appeared to be uncertain as to the analogy between freemartinism in cattle and the intersexual condition found in other species.

Sex intergrades have been observed in still other species. Hunter (*37*) described one in the ass, Crew (*9*) one in the camel, and Hartman (*34*) and Hartman and League (*35*) one in the opossum.' Many other cases of sex reversal and sex modification have been reported, several of which were in connection with studies in poultry.

The wide divergence of opinion with regard to the analogy between freemartinism in cattle and intersexuality in other species has been pointed out. Some investigators have shown placental and vascular anastomosis combined with anatomical abnormalities in a number of different species that appeared to be similar to those in cattle, and claim they are practically identical. Others, like Lush (*52*), state that the freemartin "does not appear to exist, or at least is rare among

twins in horses, sheep, goats, and other mammals." It seems probable that, if typical freemartinism does occur in other mammals it is the exception rather than the rule, whereas in cattle it is the rule when twins of both sexes are born.

HERMAPHRODITISM IN THE HUMAN

The observation of freemartinism in cattle naturally led to legendary beliefs with regard to its occurrence in humans. Some degree of hermaphroditism supposedly occurs about once in 1,000 humans. Many causes appear to exist. Young (*66*) who made an exhaustive study of the manifestations and causes of genital abnormalities in humans indicated that the condition is not associated with twinning and that it is not analogous to the freemartin condition in cattle. Simpson (*59*) found that in 113 cases of human mixed twins 103 of the women had families. When he included several cases of triplets and 1 of quadruplets the total number of women was increased to 123, of whom only 11 failed to have offspring. This ratio of unproductive individuals was slightly less than the ratio found in his study of 1,252 marriages, of which 146 were without offspring. He found also that the number of offspring was as great for women born twin with males as for women born singly. It is extremely doubtful if the human sex intergrade is analogous to the bovine freemartin, as it appears that no authentic case has ever been found.

EXPERIMENTALLY PRODUCED SEX INTERGRADES

Strong experimental evidence supporting Lillie's theory that freemartinism is caused by the influence of male hormones, has been presented recently by Greene and Ivy (*28*) and by Greene, Burrill, and Ivy (*24, 26*) who showed that testosterone or testosterone propionate (male sex hormones) when injected into pregnant rats resulted in a high frequency of abnormal females in the offspring, the abnormalities closely resembling those of the bovine freemartin. The degree of modification appeared to depend on the stage of pregnancy when injected and on the quantity of hormone administered. These authors appear to believe the condition developed is analogous to freemartinism. Raynaud (*56*), Hamilton and Gardner (*30*), and Hamilton and Wolfe (*31*) largely confirmed these results with mice and with rats. Dantchakoff (*12, 13, 14, 15, 16*) obtained similar results with guinea pigs, and showed that the hormone from the adult testicle is identical with that causing the freemartin in cattle. Greene, Burrill, and Ivy (*27*) have shown also that injections of female sex hormones into pregnant rats are capable of producing feminized males. The same authors (*25*) have found that the male sex hormone will cause masculinization when injected into the newborn rat, and Raynaud and Lacassagne (*57*) have shown that sex modification results in the mouse from the injection of either male or female hormones in the newborn or the adult.

MAMMARY DEVELOPMENT, ENDOCRINE ABNORMALITIES, AND OTHER CHARACTERISTICS OF THE FREEMARTIN

The dearth of specific references concerning mammary-gland structure in freemartins has been particularly noticeable. Those found have been of a very general nature. Hunter (*37*) described a 5-year-old freemartin in which "there were four teats, the glandular

part of the udder was but small," and a 3-to-4-year-old freemartin in which the teats and udder were small compared with those of a heifer. Scarpa in 1784, according to Ballantyne (*1*) stated that the freemartin resembled a bull but that under the abdomen were mammary glands and teats. Numan in 1829, according to Hart (*32*) described a case in which there was no trace of an udder, the skin in that region was lax and pendent like a sac, and the teats were of the same size as those of a bull. Lillie (*47*) stated that the mammary gland is almost invariably of the female type. Crew (*10*) described the mammary development of the freemartin as similar to that of the immature female and quoted Hartman and League (*35*) as crediting the freemartin with having an udder. Bissonnette (*2*) described the udder rudiments (in bovine fetuses) as apparently normal for females of such kind and size. Galli (*20*) spoke of mammary glands as often absent. Buyse (*3*) stated that no udder was present and the teats were no longer than those found ordinarily in normal bulls. Davies (*17*) referred to the teats of an intersex goat as rudimentary; Rickards and Jones (*58*) described a goat in which the nipples and mammary glands were normal, and its twin in which a scrotal pouch under the site of the mammary gland produced an elevation of the udder making it appear well-developed and normal. Hunter (*37*) mentioned a similar condition observed in an intersex horse. Curson (*11*) seems to have been the only one to go so far as to illustrate the external view of the excised udders of two freemartins. No detailed descriptions of the mammary-gland structure in the freemartin have been found.

Still less information is found regarding the endocrine glands in the freemartin. Young (*66*) and Glynn (*21*) found some associations between endocrine dysfunction and sex malformations in the human. Davies (*17*) stated that the adrenals and thyroid of an intersex goat were undisturbed; Hart (*33*) found that the thyroid, thymus, and suprarenals of a freemartin were normal; and Crew (7) found no endocrine disturbance in well-grown intersexual animals, except in the sex glands.

References to the body form of freemartins are few in number. Keller (*40*) showed that freemartins were taller in relation to length than normal heifers, resembling spayed heifers in this respect; that the increased height was due almost entirely to lengthening of the long bones, chiefly the metacarpus; and that freemartins showed prominent withers resulting from elongation of the spinous processes of the vertebrae, a greater horn development, and peculiarities in pelvic form. Numan in 1829, according to Hart (*32*), described a freemartin as resembling an ox in conformation with long, narrow head and long, wide horns. He also said that it had a large, broad, high body, with small bones, and stood high on its limbs. Ballantyne (*1*) referred to a letter in which a farmer expressed existing ideas by describing a certain woman as "just like a freemartin, and had no hips at all." Fincher and Williams (*18*) referred to a freemartin that was slow in developing, and that attained only 70 percent normal size after 1 year.

The enlarged clitoris has often been mentioned in connection with the freemartin. Williams (*65*) showed that, whereas both the female and the male in cattle assume a characteristic pose when urinating, the neuter assumes no such pose beyond the elevation of

the tail. Hunter (*37*) described the bellow of the freemartin as similar to that of the ox, and not at all like that of the bull. Davies (*17*) stated that the caprine freemartin assumed a male position when standing, but that the shape of the head and body resembled the female. Other observers of intersex goats have commented on their habits and the fact that their appearance became increasingly masculine with advance in age. Fincher and Williams (*18*) failed to find typical escutcheons in the bovine asexuals studied and suggested that deviations in the escutcheon may signify teratological aberrations in the internal female genitalia.

PROPORTION OF NORMAL BREEDERS AMONG THE FEMALES OF MIXED TWINS IN CATTLE

Although it has long been recognized that occasionally a female born twin with a bull is normal sexually and therefore is not a freemartin, data are exceedingly fragmentary with regard to the frequency with which such normal cases occur. Strebel (*61*) reported finding 1 normal female among 8 females twinborn with bulls, indicating that 12.5 percent were normal. It is noteworthy that this fertile female gave birth to a sterile heifer. Williams (*65*) indicated that the frequency of normal cases is approximately 1 in 7; Spencer (*60*) referred to 11 cases of which none was capable of reproduction; and Lillie (*51*) summarized his own data and those of others as follows:

Source of data:	*Ratio*
Keller and Tandler (6 normals in 91)	1:15.2
Lillie (6 normals in 39)	1:6.5
Luer (6 normals in 113)	1:18.8
Numan	1:8.0

The average of the 4 ratios is 1:12.1 meaning that a normal female might be expected once in about 12 times. For the 243 cases in the first 3 groups the ratio is 1:13.5 which means that 7.4 percent of the females born twin with bull calves were normal sexually. It is noteworthy that Lillie's data, which show an exceptionally high ratio of normal to abnormal females, were based on histological studies of embryos and that none ever had an opportunity to prove its ability to become a normal breeder.

PLAN OF STUDY AND PROCEDURE FOLLOWED

In connection with a continuous study at Beltsville, Md., of the relation of the conformation and anatomy of the dairy cow to her producing capacity, the authors have made a detailed analysis of the growth, conformation, anatomy, and mammary structure of the viable females that were born twin to bulls in the Bureau's herd. These female twins were slaughtered at different ages in order to provide a basis for comparing them, at different stages of development, with females that were born singly and that presumably were essentially normal in conformation, anatomy, and sexual development.

The data obtained on growth and conformation consist of the live weight and a large number of body measurements for each animal. The anatomical data include weights and/or measurements of nearly all the internal organs of the body—including endocrine glands, and measurements of the thoracic cavity.

Consideration was given to abnormalities in both the external and internal genitalia. Studies of the udder included comparisons of the degree of mammary-gland development in individual animals at

different ages, and of the structure of the mammary tissue at the time of slaughter.

In view of the large number of freemartins that have been described in detail since the notable work of Hunter (*37*), a complete case history of all the cases on which this study is based is not justifiable. In general the cases studied have followed closely the pattern of those described by others. Wide variations in degree of sex modification have been found. The individual animals have had the external genitalia of the female—with slight modifications in some cases—and the physical appearance of females. The internal genitalia have in most cases been vestigial and in some cases what appeared to be male gonads were present. In one case one testicle was found at a point close to the usual location of the ovary and one descended and lodged on the upper surface of the udder (pl. 1, *A*, *B*). So far as the genitalia are concerned every case observed seems to have fallen within the limits of modification reported by others. In this report no attempt is made to show the genital abnormalities of all the freemartins studied. A single comparison of the internal genitals of freemartin No. 1006 at 18 months 2 days of age (pl. 1 *A*, B,) with those of a normal heifer at 16 months of age (pl. 2) will suffice to show the extent to which a freemartin was found to depart from the normal. The chief purpose of this report is to furnish additional data on certain characteristics of the freemartin that have been discussed by others, and to present results on other points that other investigators have treated lightly or not at all.

ANIMALS STUDIED

Seventeen females born twin with bull calves were studied. Seven were Jerseys, eight were Holstein-Friesians and two were grade Holsteins. Six were slaughtered at approximately 18 months of age, one was slaughtered at 15 months, two at 12 months, one at 9 months, one at 6 months, two at 3 months, and two at 2 months. The other two died at less than 1 month of age.

Table 1 lists the animals studied and shows the breed, the age at death or slaughter, and the herd number and reproduction record of the dam.

TWIN-PRODUCING TENDENCIES IN INDIVIDUAL COWS

There is much in the literature to indicate that, in species that ordinarily bear 1 offspring at each parturition, the tendency to produce twins may be inherited. In this connection some observations with regard to the data in table 1 are noteworthy. Cow No. 667 was herself born twin with a female, and in 8 calvings gave birth to twins of unlike sex 3 different times. One of her female calves born twin with a bull was aborted; the other 2 appear among the freemartins listed (Nos. 1421 and 1485). Cow No. 289 gave birth to twins at 3 different parturitions; at the fourth calving she dropped twin heifers, and at the sixth and seventh calvings twins of unlike sex were born, the females being listed as Nos. 1200 and 1229. Moreover, the autopsy of No. 289 showed that she was carrying twin female fetuses, making her fourth set of twins, 3 of which occurred at successive pregnancies. The fact that 5 of the dams (Nos. 485, 460, 271, 667, and 289) 1 of which was herself a twin, gave birth to 13 sets of twins, might be interpreted as indicating that twinning was an inherited trait in these animals.

TABLE 1.—*Females born twin with male calves, and reproduction record of their dams*

Herd No. of female twin	Breed	Age at death or slaughter	Herd No. of dam	Dam's age when female twin was born	Dam's parturition when twin was born	Sex of dam's offspring at each parturition—									Remarks
						First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	
		Yr. Mo. Day		*Yr. Mo. Day*											
91	Jersey	1 6 25	485	7 8 10	Fifth	M	M	M	M	M-F	F	M	M		Carrying mixed twin fetuses at death.
34	Holstein	1 6 16	290	4 1 3	Second	F [1]	M-F	M	M						
24	Jersey	1 6 3	460	6 8 6	Fifth [2]	(3)	(3)	F	M	M-F	F	(4)	M	M [5]	
006	do	1 6 2	628	4 0 6	Second	F	M-F	F	F	F	M	M			
-70	Grade Holstein	1 5 29	A-49	2 0 24	First	M-F									Carrying female fetus at death.
96	Holstein	1 5 23	229	8 8 4	Sixth	F [1]	M	F [5]	M	M	M-F				
-82	Grade Holstein	1 2 28	A-37	4 11 24	Third	M	F	M [6]-F							
209	Holstein	1 0 11	271	11 0 22	Ninth	M	M	(7)	F	F	M	M [1]	F-F	M [6]-F	
421	Jersey	1 0 6	667	6 11 13	Fifth	M [5]-F [5]	F	F	F	M-F	M	M	M-F		
06	Holstein	0 8 22	274	3 6 7	Second	M	M-F	M	F	M					
427	Jersey	0 6 1	670	6 7 4	Fifth	F	M	F	M [5]	M [6]-F	M				
229	Holstein	0 3 0	289	9 8 2	Seventh	M	F	F	F-F	F	M-F	M-F			Carrying female twin fetuses at death.
212	do	0 2 28	841	4 2 27	Third	F	M	M-F							
485	Jersey	0 2 1	667	9 11 15	Eighth	M [1]-F [5]	F	F	F	M-F	M	M	M-F		
205	Holstein	0 2 0	821	5 0 24	Third	F [1]	M	M-F	F	F	M	F	M		
408	Jersey	0 0 26	1059	2 3 19	First	M-F	(8)	M	M	F	M				
200	Holstein	0 0 18	289	8 6 20	Sixth	M	F	F	F-F	F	M-F	M-F			Do.

[1] Born dead or died at birth (268 to 290 days' gestation).
[2] *Estimated on basis of dam's age at first calving after purchase.*
[3] Breeding record not available.
[4] Aborted twins.
[5] Aborted.
[6] Male of twins of unlike sex born dead (270 to 283 days' gestation).
[7] Mummified fetus.
[8] Resorbed fetus.

AGE OF DAM AT THE BIRTH OF FREEMARTINS

Many investigators have found that the incidence of twinning increases with the age of the mother up to a fairly advanced age. Of the 17 females born twin with males (table 1), 2 were born of 2-year-old dams, 1 of a 3-year-old dam, 4 of 4-year-old dams, 1 of a 5-year-old dam, 3 of 6-year-old dams, 1 of a 7-year-old dam, 2 of 8-year-old dams, 2 of 9-year-old dams, and 1 of an 11-year-old dam. Eight were born of dams less than 6 years old, and 9 of dams over 6 years. Counting all twin conceptions on record for the 15 dams listed in table 1, including the twin fetuses carried by 2 of the cows at death, the occurrence of twins at each gestation from the first to the ninth inclusive is 3, 3, 3, 1, 4, 2, 2, 3, and 2. The data presented do not appear to indicate any particular age at which twins of either unlike or like sex are most likely to occur. It is noteworthy, however, that not all the cows lived to have 9 conceptions, although 7 of the 15 had 7 or more. If all had continued breeding through 9 conceptions there might have been a shifting of the incidence of twinning toward a more advanced age. Moreover, the results are based on a highly selected group of dams.

PLACENTAL AND VASCULAR ARRANGEMENT

The data obtained in connection with the twins of unlike sex on which this report is based are too limited to be positively identified as supporting or contradicting the theories propounded by Lillie. It is very difficult to make accurate observations on placental and vascular arrangement after the birth of twin calves, and such data were obtainable in only four cases. The observations in connection with these four cases, as made by an experienced veterinary physiologist, are as follows: No. A–70, a sexually abnormal female, was carried in an entirely separate placenta from that of its male twin, but it was not definitely determined whether or not vascular connection existed; in the case of No. 1209 there were separate placentae connected by blood vessels; in the case of No. 1229 the blood vessels from each fetus led to the same cotyledons but did not otherwise anastomose; and in the case of No. 1408 which was determined on autopsy to be normal sexually, each fetus was in a separate placenta and there was no connection between them.

Autopsies of two pregnant cows in the Beltsville herd (A–40 and 485) provided two sets of twin fetuses of unlike sex for observation. In the case of A–40, both of the 233-day fetuses were in the same placenta and there was anastomosis of the blood vessels. One fetus was a male, and the other was determined to be a neuter. The 131-day twin fetuses obtained from cow No. 485 on autopsy were also in a common placenta and there was anastomosis of the blood vessels. The twins were male and female so far as external manifestations were concerned; but as the fetuses and the placental membranes were preserved unseparated, the matter of sexual normalcy of the female was not determined. Plate 3 shows the placental membranes from cow No. A–40 and the membranes and fetuses from cow No. 485, both of which show vascular fusion. The findings reported do not appear to be contrary to the theory advanced by Lillie except possibly in the case of A–70, for which there is nothing in the notes to indicate that vascular fusion was present.

RELATIVE WEIGHT AND CONFORMATION OF THE FREEMARTIN

Only a few general statements have been found in the literature that refer to the size and external form of freemartins as compared with females of the same breed that were born singly. A summary of the weights and body measurements of the freemartins included in this study is given in table 2. The breed averages shown were determined specifically for this study from data obtained over a period of approximately 14 years on females in the Beltsville herd. In order to minimize the tabulation work, the breed averages were determined on the basis of every tenth animal having weights and body measurements, at ages ranging from 3 to 18 months, inclusive. The sample groups so formed contained 17 Holstein-Friesians and 20 Jerseys.

TABLE 2.—*Live-weight and body measurements of freemartins, and of heifers of single births, at 3 to 18 months of age*

Item of comparison, and age of animal (months)	Jerseys			Holsteins				
					Registered parents		Grade parents	
	Breed average	Freemartin average	Relation to breed average	Breed average	Freemartin average	Relation to breed average	Freemartin average	Relation to breed average
Live weight:	*Pounds*	*Pounds*	*Percent*	*Pounds*	*Pounds*	*Percent*	*Pounds*	*Percent*
3	146	130	89.0	211	175	82.9	142	67.3
6	284	248	87.3	396	334	84.3	296	74.7
12	518	479	92.5	698	608	87.1	581	83.2
18	663	590	89.0	899	761	84.6	720	80.1
Average			89.5			84.7		76.3
Height at withers:	*Centimeters*	*Centimeters*	*Percent*	*Centimeters*	*Centimeters*	*Percent*	*Centimeters*	*Percent*
3	81.62	79.65	97.6	87.75	83.18	94.8	77.17	87.9
6	95.77	93.63	97.8	102.03	98.98	97.0	94.67	92.8
12	110.35	110.40	100.0	117.82	118.03	100.2	111.75	94.8
18	117.35	118.86	101.3	126.71	127.04	100.3	118.50	93.5
Average			99.2			98.1		92.
Height at hips:								
3	84.43	81.63	96.7	92.02	88.10	95.7	78.88	85.7
6	98.41	94.58	96.1	106.68	104.77	98.2	96.17	90.1
12	112.66	110.46	98.0	122.66	123.00	100.3	114.25	93.1
18	119.06	118.42	99.5	130.85	132.25	101.1	120.42	92.0
Average			97.6			98.8		90.2
Height at pin bones:								
3	83.55	80.97	96.9	91.68	86.57	94.4	78.33	85.4
6	97.13	93.43	96.2	105.27	102.00	96.9	95.58	90.8
12	110.68	109.42	98.9	120.50	118.03	98.0	112.09	93.0
18	116.70	116.64	99.9	128.29	126.96	99.0	119.92	93.5
Average			98.0			97.1		90.7
Length (shoulder to pin bones):								
3	84.95	82.41	97.0	92.41	85.19	92.2	78.94	85.4
6	103.62	100.13	96.6	114.27	105.88	92.7	105.19	92.1
12	127.27	124.04	97.5	138.17	131.51	95.2	127.69	92.4
18	139.36	133.82	96.0	152.61	141.00	92.4	141.13	92.5
Average			96.8			93.1		90.6
Total length, withers to pin bones:								
3	69.66	68.63	98.5	75.58	70.25	92.9	67.50	89.3
6	87.50	84.44	96.5	93.42	90.83	97.2	86.75	92.9
12	106.17	104.25	98.2	113.05	111.00	98.2	107.50	95.1
18	114.71	112.08	97.7	125.17	119.00	95.1	113.00	90.3
Average			97.7			95.9		91.9

ABLE 2.—*Live-weight and body measurements of freemartins, and of heifers of single births, at 3 to 18 months of age*—Continued

Item of comparison, and age of animal (months)	Jerseys			Holsteins				
					Registered parents		Grade parents	
	Breed average	Free-martin average	Relation to breed average	Breed average	Free-martin average	Relation to breed average	Free-martin average	Relation to breed average
	Centimeters	*Centimeters*	*Percent*	*Centimeters*	*Centimeters*	*Percent*	*Centimeters*	*Percent*
ength of loin:								
3	18.75	18.50	98.7	19.88	18.55	93.3	18.00	90.5
6	23.84	23.25	97.5	25.25	23.63	93.6	23.25	92.1
12	27.98	27.63	98.7	29.22	28.83	98.7	29.63	101.4
18	29.99	29.83	99.5	31.68	31.13	98.3	30.00	94.7
Average			98.6			96.0		94.7
epth fore chest:								
3	35.61	34.80	97.7	38.34	36.17	94.3	33.75	88.0
6	44.53	42.77	96.0	47.29	45.06	95.3	43.50	92.0
12	54.93	54.17	98.6	58.65	57.03	97.2	55.13	94.0
18	60.34	59.72	99.0	64.97	62.88	96.8	58.25	89.7
Average			97.8			95.9		90.9
epth rear chest:								
3	35.51	34.35	96.7	38.60	35.92	93.1	33.25	86.1
6	44.99	43.60	96.9	47.90	45.88	95.8	43.75	91.3
12	55.25	54.00	97.7	58.71	56.78	96.7	56.38	96.0
18	59.59	58.56	98.3	63.79	62.63	98.2	57.92	90.8
Average			97.4			96.0		91.1
epth paunch:								
3	36.98	35.50	96.0	40.57	38.10	93.9	35.88	88.4
6	46.55	44.73	96.1	50.16	47.50	94.7	47.25	94.2
12	56.41	54.44	96.5	60.40	59.06	97.8	58.75	97.3
18	60.20	58.44	97.1	64.60	63.50	98.3	59.58	92.2
Average			96.4			96.2		93.0
Width of fore chest:								
3	18.35	16.72	91.1	21.83	19.70	90.2	17.75	81.3
6	23.72	21.95	92.5	27.91	25.33	90.8	24.13	86.5
12	31.20	29.02	93.0	35.94	32.22	89.6	33.63	93.6
18	34.80	32.78	94.2	40.28	36.46	90.5	35.50	88.1
Average			92.7			90.3		87.4
Width of rear chest:								
3	25.60	24.40	95.3	30.28	27.85	92.0	26.63	87.9
6	33.92	32.98	97.2	39.28	37.77	96.2	33.84	86.2
12	44.08	41.79	94.8	49.34	47.05	95.4	45.67	92.6
18	48.87	45.36	92.8	53.38	49.92	93.5	47.67	89.3
Average			95.0			94.3		89.0
Width of paunch:								
3	27.73	27.12	97.8	32.72	30.99	94.7	29.46	90.0
6	37.70	36.83	97.7	43.53	41.69	95.8	38.84	89.2
12	49.00	47.00	95.9	54.06	51.86	95.9	53.00	98.0
18	53.75	49.61	92.3	57.76	53.29	92.3	52.92	91.6
Average			95.9			94.7		92.2
Width of hips:								
3	20.83	20.00	96.0	24.32	21.75	89.4	20.25	83.3
6	27.74	26.80	96.6	31.06	29.31	94.4	28.13	90.6
12	37.14	36.06	97.1	40.54	38.17	94.2	37.38	92.2
18	41.53	39.50	95.1	46.35	41.88	90.4	42.00	90.6
Average			96.2			92.1		89.2
Width of pin bones:								
3	13.20	11.70	88.6	16.69	14.96	89.6	12.63	75.7
6	16.71	15.40	92.2	21.47	19.69	91.7	18.25	85.0
12	22.59	21.13	93.5	28.60	25.67	90.1	23.88	83.8
18	25.65	23.00	89.7	33.32	29.00	87.0	30.25	90.8
			[illegible]			[illegible]		[illegible]

TABLE 2.—*Live-weight and body measurements of freemartins, and of heifers single births, at 3 to 18 months of age*—Continued

Item of comparison, and age of animal (months)	Jerseys			Holsteins				
					Registered parents		Grade parent	
	Breed average	Freemartin average	Relation to breed average	Breed average	Freemartin average	Relation to breed average	Freemartin average	Relation to breed avera
	Centimeters	*Centimeters*	*Percent*	*Centimeters*	*Centimeters*	*Percent*	*Centimeters*	*Perce*
Width of thurls:								
3	22.63	21.65	95.7	26.91	24.96	92.8	21.63	80
6	28.69	27.00	94.1	33.62	32.00	95.2	29.50	87
12	35.24	34.00	96.7	41.29	39.42	95.5	37.63	91
18	38.56	36.67	95.1	45.74	43.13	94.3	41.25	90
Average			95.4			94.5		87
Width of loin:								
3	14.44	14.40	99.7	16.87	15.54	92.1	14.25	84
6	19.09	18.80	98.5	21.52	20.25	94.1	19.13	88
12	24.93	24.56	98.5	27.97	27.00	96.5	24.88	89
18	27.63	27.58	99.8	31.88	30.00	94.1	27.00	84
Average			99.1			94.2		86
Circumference of fore chest:								
3	91.35	87.00	95.2	101.94	95.17	93.4	87.25	85
6	113.88	108.90	95.6	125.59	118.75	94.6	113.00	90
12	142.75	139.63	97.8	155.85	148.83	95.5	147.00	94
18	156.98	151.00	96.2	172.41	164.00	95.1	155.50	90
Average			96.2			94.7		90
Circumference of rear chest:								
3	102.30	97.80	95.6	115.88	106.75	92.1	105.50	91
6	130.23	125.60	96.4	143.65	135.50	94.3	128.25	89
12	161.53	156.00	96.6	176.12	168.83	95.9	167.00	94.
18	175.63	167.67	95.5	190.79	180.50	94.6	177.00	92
Average			96.0			94.2		92.
Circumference of paunch:								
3	109.55	106.00	96.8	123.62	116.25	94.0	114.00	92.
6	139.78	135.90	97.2	155.15	147.13	94.8	143.00	92.
12	171.15	166.75	97.4	186.44	180.67	96.9	181.50	97.
18	184.08	175.00	94.8	198.47	187.75	94.0	187.00	94.
Average			96.6			95.1		94.
Length of head (Caliper):								
3	27.77	27.06	97.4	31.33	29.70	94.8	27.63	88.
6	33.52	32.38	96.6	37.03	36.17	97.7	34.13	92.
12	39.88	40.00	100.3	44.06	44.00	99.9	41.25	93.
18	43.18	42.58	98.6	48.25	46.00	95.3	44.25	91.
Average			98.2			96.9		91.
Width of forehead (Caliper):								
3	12.03	12.44	103.4	12.77	12.00	94.0	11.75	92.
6	13.56	14.19	104.6	14.98	13.92	92.9	14.00	93
12	15.26	16.92	110.9	16.91	16.13	95.4	16.38	97.
18	16.13	17.58	109.0	17.86	16.75	93.8	16.50	92.
Average			107.0			94.0		93
Width of head, across eyes:								
3	13.96	13.38	95.8	15.14	14.00	92.5	13.00	85.
6	16.46	15.94	96.8	17.77	16.38	92.2	16.25	91.
12	19.08	18.58	97.4	20.50	19.38	94.5	19.63	95.
18	20.64	19.88	96.3	21.87	20.75	94.9	20.75	94.
Average			96.6			93.5		92.
Circumference of shinbone:								
3	10.29	9.50	92.3	12.26	11.38	92.8	10.38	84.
6	12.15	11.50	94.7	14.24	13.50	94.8	12.88	93.
12	14.43	14.50	100.5	16.69	16.17	96.9	15.38	92.
18	15.33	15.00	97.8	18.03	17.50	97.1	16.50	91.
Average			96.3			95.4		[illegible]

The extent to which the freemartins differed from the breed averages is more clearly indicated in table 3, which gives the distribution of the average percentages shown in table 2 that represent the proportionate development of the freemartins to that of the breed average.

TABLE 3.—*Distribution of percentages that show the relation of the freemartins to the average for the respective breed*

Breed	Items of body size or measurement in which the relation of the freemartin average to the breed average was—				
	100 percent or above	95.0 to 99.9 percent	90.0 to 94.9 percent	85.0 to 89.9 percent	Below 85 percent
	Number	*Number*	*Number*	*Number*	*Number*
Jersey	1	20	2	1	----------
Holstein:					
Registered	----------	11	11	1	1
Grade	----------	----------	16	6	2

In general the Jersey freemartins were not greatly retarded in development; the Holstein freemartins were definitely undersized; and the grade Holstein freemartins were very much below the Holstein breed average in body size. Undersize was most pronounced in live weight. Apparently, however, the comparatively low weights and measurements of the freemartins did not result from condition of flesh. Deficiency in body size in freemartins seemed to be more pronounced in widths than in the other body dimensions. In other words the freemartins were small, and they were inclined to be narrow in relation to height, length, and depth. The undersize of the grade Holstein freemartins is probably not significant since the grade Holsteins in the Beltsville herd are intensely inbred and the whole population is undersized.

COMPARISON OF FREEMARTINS WITH HEIFERS TWINNED WITH HEIFERS

Since twins usually are below the average in weight at birth the question arose as to whether the freemartins under consideration were undersized because they were freemartins or because they were twins. The twin heifers in the herd that lived to at least 12 months of age were compared with the breed averages used in studying the comparative development of freemartins. Data were available for five Jerseys and two Holsteins. All but one of the seven were measured up to at least 18 months of age.

In live weight the Jersey twin heifers were definitely smaller than the Jersey freemartins at 3 months but had attained greater size than the freemartins at 6 months and remained heavier both at 12 and 18 months of age. However, they did not reach the breed average, the percentages of average weight at 3, 6, 12, and 18 months being 81.5, 91.2, 93.6, and 92.6, respectively; the comparative percentages for the freemartins were 89.0, 87.3, 92.5, and 89.0. For the 23 measurements of body size the average percentages at 3, 6, 12, and 18 months were 93.9, 96.9, 96.4, and 96.7 for the twin heifers as compared with 96.4, 96.5, 98.0, and 97.2 for the freemartins. In skeletal growth both groups were within about 6 percent of the breed average at 3 months

The two Holstein twins were not weighed or measured until they were 6 months old. They were smaller at 6 months of age than the Holstein freemartins studied. This was especially true for live weight and occurred also for 19 of the 23 items of body measurement. There was a definite tendency for the Holstein twins to approach the breed average with advance in age—nearly or entirely reaching it at 18 months, while the Holstein freemartins remained approximately as far below the breed average at 18 months as at 6 months of age.

Of course, definite conclusions cannot be based on such small groups of animals. Taking both breeds into consideration, however, it appears that not only were the freemartins definitely undersized at the ages represented, but there was a tendency for them to approach the breed average more slowly than heifers twinned with heifers—especially as far as live weight is concerned. It seems probable that the smallness of the freemartins was due to the fact that they were twinborn rather than that they were freemartins.

ANATOMICAL STRUCTURE OF THE FREEMARTIN

Aside from detailed descriptions of the genitalia there is little in the reports of other investigators to show the extent to which the internal anatomy of freemartins departs from the normal. In the present study a comparison of the internal anatomy of freemartins with normal animals of the same breed and age was difficult because of the limited data available on normal animals. The anatomy of the freemartins slaughtered at 2 and 3 months of age was compared with that of bulls because data were not available for normal heifers of these ages. It was necessary also to combine the data for Holsteins with those for Jerseys in some of the groups. Resulting inconsistencies are overcome for the most part by the fact that, except for age and empty-body weight, all comparisons are based on units of weight, or measurements, per 100 pounds empty-body weight rather than on the absolute weights, or measurements, of the organs and body parts compared. Empty-body weight is the difference between the live weight of the animal before slaughter and the weight of the contents of the digestive tract.

Table 4 shows the size of the internal organs and body parts of freemartins and of other dairy cattle of similar age, compared on a basis of the number of units of weight or measurement per 100 pounds of empty-body weight.

The individual percentages which show the relation between the freemartins and the animals used for comparison vary considerably for the different items, and for the same item at different ages. The averages shown in table 4 are based on the individual percentages rather than on the number of pounds, grams, or centimeters per 100 pounds empty-body weight because the latter are not entirely comparable. The average of the percentages for all of the ages represented is given for each item in the last column. The percentage for empty-body weight is only 78.6, which shows that the freemartins were actually much smaller than the animals with which they were compared. In view of the retarded mammary development of the freemartins it is noteworthy that the lowest percentage (81.8) is for weight of udder.

of weight or measurement per 100 pounds of empty body weight

Weight or measurements	Unit	2-month group: Freemartins [1]	2-month group: Animals used for comparison [2]	2-month group: Relation of freemartins to animals used for comparison	3-month group: Freemartins [3]	3-month group: Animals used for comparison [4]	3-month group: Relation of freemartins to animals used for comparison	6-month group: Freemartins [5]	6-month group: Animals used for comparison [6]	6-month group: Relation of freemartins to animals used for comparison	9-month group: Freemartins [7]	9-month group: Animals used for comparison [8]	9-month group: Relation of freemartins to animals used for comparison	12-month group: Freemartins [9]	12-month group: Animals used for comparison [10]	12-month group: Relation of freemartins to animals used for comparison	15-month group: Freemartins [11]	15-month group: Animals used for comparison [12]	15-month group: Relation of freemartins to animals used for comparison	18-month group: Freemartins [13]	18-month group: Animals used for comparison [14]	18-month group: Relation of freemartins to animals used for comparison	Average all ages: Relation of freemartins to animals used for comparison
		No.	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*Pct.*
ıpty-body weight	Pounds [15]	83.00	107.71	77.1	144.48	189.50	76.2	169.05	259.74	65.1	356.85	391.00	91.3	421.37	516.39	81.6	324.45	[illegible]	[illegible]	569.19	563.10	101.1	78.6
·e weight	do	112.36	108.94	103.1	112.55	110.82	101.6	117.13	115.38	101.5	135.63	116.37	116.6	114.68	114.64	100.0	118.66	[illegible]	[illegible]	113.46	116.30	97.6	103.2
ight of blood	do	5.24	5.02	104.4	6.22	5.80	107.2	6.09	5.13	118.7	3.50	5.24	66.8	4.45	4.27	104.2	4.59	[illegible]	[illegible]	4.18	4.56	91.7	100.4
ight of thyroid	Grams	8.66	8.07	107.3	10.24	13.56	75.5	5.92	5.19	114.1	6.22	6.14	101.3	4.89	3.98	122.9	4.44	[illegible]	[illegible]	4.24	3.65	116.2	111.8
ight of brain	do	301.73	259.68	116.2	199.29	178.36	111.7	148.48	122.85	120.9	92.48	99.74	92.7	85.89	72.60	118.3	98.63	[illegible]	[illegible]	68.04	66.78	101.9	114.2
ight of pituitary	do	.68	.72	94.4	.61	.61	100.0	.51	.44	115.9	.42	.36	116.7	.32	.30	106.7	.42	[illegible]	[illegible]	.33	.33	100.0	112.0
ight of udder	Pounds	.59	----	----	.85	----	----	.92	.90	102.2	.66	.93	71.0	1.25	1.52	82.2	.62	[illegible]	[illegible]	1.76	1.60	110.0	81.8
ight of hide	do	8.34	9.37	89.0	7.70	9.13	84.3	8.49	8.67	97.9	7.85	8.93	87.9	8.80	8.54	103.0	11.59	[illegible]	[illegible]	7.78	8.96	80.8	99.3
ight of liver	do	2.16	1.87	115.5	2.17	1.98	109.6	2.54	1.98	128.3	1.75	1.80	97.2	1.63	1.71	95.3	2.42	[illegible]	[illegible]	1.44	1.52	94.7	112.6
ight of spleen	Grams	173.10	155.88	111.1	124.87	165.44	75.5	100.86	108.64	92.8	88.97	124.17	71.7	99.84	95.88	104.1	69.66	[illegible]	[illegible]	102.93	103.04	99.9	88.8
ight of pancreas	do	49.63	41.86	118.6	56.76	43.43	130.7	52.06	54.14	96.2	46.80	37.85	123.7	54.27	46.11	117.7	42.84	[illegible]	[illegible]	60.32	46.40	130.0	116.0
tal length of intestines	Feet	117.44	84.15	139.6	76.35	62.30	122.6	81.51	53.31	152.9	41.73	40.55	102.9	33.88	29.76	113.8	41.77	[illegible]	[illegible]	26.55	29.22	90.9	123.6
tal weight of empty ıtestines.	Pounds	6.68	4.58	145.9	4.36	4.49	97.1	5.65	4.04	139.9	4.06	3.21	126.5	2.32	2.36	98.3	3.11	[illegible]	[illegible]	2.48	2.40	100.8	118.8
ight of rumen	do	1.01	.89	113.5	1.18	1.32	89.4	2.19	1.82	120.3	2.28	1.76	129.6	1.66	1.63	101.8	1.74	[illegible]	[illegible]	1.74	1.77	98.3	107.2
ight of reticulum	do	.21	.23	91.3	.23	.26	88.5	.47	.26	180.8	.29	.27	107.4	.23	.24	95.8	.28	[illegible]	[illegible]	.31	.29	106.9	110.6
ight of omasum	do	.23	.22	104.6	.35	.45	77.8	.98	.80	122.5	1.15	.90	127.8	.86	.98	87.8	.83	[illegible]	[illegible]	.95	1.00	95.0	99.8
ight of abomasum	do	.55	.51	107.8	.53	.47	112.8	.65	.41	158.5	.46	.40	115.0	.37	.36	102.8	.71	[illegible]	[illegible]	.45	.41	109.8	129.1
tal weight of stomachs	do	2.00	1.85	108.1	2.28	2.51	90.8	4.29	3.30	130.0	4.19	3.32	126.2	3.12	3.20	97.5	3.56	[illegible]	[illegible]	3.44	3.47	99.1	108.0
tal weight of lungs	do	1.40	1.55	90.3	1.64	1.35	114.1	1.36	1.04	130.8	.91	.97	93.8	.96	.86	111.6	1.34	.86	[illegible]	.83	.86	96.5	113.3
ight of heart	do	.60	.64	93.8	.55	.61	90.2	.56	.47	119.2	.53	.47	112.8	.47	.41	114.6	.54	.37	146.0	.37	.39	94.9	110.2
ight of kidneys	Grams	221.61	236.77	93.6	223.84	238.89	93.7	212.36	194.85	109.0	139.83	135.29	103.4	128.75	129.54	99.4	195.41	113.85	171.6	104.38	110.02	94.9	109.4
ight of adrenals	do	3.54	4.26	83.1	3.66	4.01	91.3	4.08	3.11	131.2	2.44	2.40	101.7	2.44	2.65	92.1	3.48	2.45	142.0	2.26	2.47	91.5	104.7

See footnote at end of table.

ABLE 4.—*Size of internal organs and body parts of freemartins and of other dairy cattle of similar age, compared on basis of number of units of weight or measurement per 100 pounds of empty-body weight*—Continued

Weight or measurements	Unit	2-month group: Freemartins	2-month group: *Animals used for comparison*	2-month group: Relation of freemartins to animals used for comparison	3-month group: Freemartins	3-month group: Animals used for comparison	3-month group: Relation of freemartins to animals used for comparison	6-month group: Freemartins	6-month group: Animals used for comparison	6-month group: Relation of freemartins to animals used for comparison	9-month group: Freemartins	9-month group: Animals used for comparison	9-month group: *Relation of freemartins to* animals used for comparison	12-month group: *Freemartins*	12-month group: *Animals used for comparison*	12-month group: Relation of freemartins to *animals used for comparison*	15-month group: Freemartins	15-month group: Animals used for comparison	15-month group: Relation of freemartins to animals used for comparison	18-month group: Freemartins	18-month group: Animals used for comparison	18-month group: Relation of freemartins to animals used for comparison	Average all ages: Relation of freemartins to animals used for comparison
		No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
eight of dressed carcass	Pounds	55.51	61.57	90.2	60.11	60.16	99.9	53.45	59.18	90.3	61.51	60.91	101.0	59.89	62.64	95.6	56.63	64.03	88.4	61.65	61.33	100.5	95.1
pth of thoracic cavity at *eighth thoracic vertebra*.	Centimeters	26.46	20.98	126.1	16.65	13.19	126.2	17.01	11.33	150.1	9.11	8.18	111.4	9.12	7.45	122.4	11.79	7.26	162.4	7.25	7.50	96.7	127.9
aximum length of thoracic cavity.	do	41.69	33.92	122.9	28.53	23.61	120.8	27.95	19.48	143.5	15.83	14.10	111.6	13.09	11.77	111.2	18.80	11.84	158.8	11.16	11.28	98.9	124.0
idth of thoracic cavity at seventh rib.	do	17.99	14.93	120.5	13.18	10.40	126.7	10.83	8.50	127.4	7.12	6.32	112.7	5.92	5.18	114.3	8.23	5.28	155.9	5.00	5.16	96.9	122.1
eight of thymus	Grams	119.27	109.04	109.4	212.69	118.10	180.1	162.38	100.42	161.7	51.56	103.84	49.7	107.57	88.24	121.9	8.66	88.16	9.8	53.66	86.70	61.9	99.2
eight of pineal	do	*.19*			.11	.08	137.5	.08	.06	133.3				.04	*.04*	*100.0*	.47	.57	82.5	.05	.04	125.0	115.7
Average all items [16]	Percent			109.0			108.8			123.0			99.2			106.7			122.1			99.1	109.8

[1] 1 Holstein and one Jersey, average age 2 months 1 day.
[3] 3 bulls (2 Holsteins, and 1 Jersey), average age 1 month 27 days.
[3] 2 Holsteins, average age 2 months, 29 days.
[4] 1 Holstein bull, age 3 months 2 days.
[5] 1 Jersey, age 6 months 1 day.
[6] 4 *heifers (2 Holsteins and 2 grade Holsteins)*, average age 6 months 1 day.
[7] 1 Holstein, age 8 months 22 days.
[8] 1 Holstein heifer, age 9 months 2 days.
[9] 1 Holstein and 1 Jersey, average age 12 months 9 days.
[10] 4 heifers (1 Holstein and 3 grade Holsteins), average age 12 months.
[11] 1 grade Holstein, age 14 months 28 days.
[12] 3 grade Holstein heifers, average age 15 months.
[13] 1 Holstein, 1 grade Holstein, and 2 Jerseys, average age 18 months 11 days.
[14] 4 heifers (2 grade Holsteins and 2 Jerseys), average age 17 months 26 days.
[15] *Actual values, whereas other items are based on units per 100 pounds empty body* weight.
[16] All items except age, empty-body weight, live weight, and the individual divisions of the stomach.

Vith few exceptions the percentages are above 100. The average of he percentages for all items and for all ages is 109.8. Although the rgans and body parts of the freemartins were relatively about 10 ›ercent larger than those of the animals with which they were ompared a separate tabulation shows that actually, on an aver-ge, they were only 85.7 percent as large. In other words, the rgans of the freemartins were actually smaller but they represented greater percentage of the empty-body weight.

THE PITUITARY BODY

A study of the freemartin would be incomplete without giving some onsideration to the pituitary body in view of its important role in onnection with the functions of reproduction and lactation. The ituitary body was actually smaller in the freemartins than in the nimals used for comparison at all of the ages represented except 9 ıonths. However, table 4 shows that at 2 months the pituitary ody was smaller in relation to empty-body weight in the freemartins han in the animals with which they were compared, whereas at months there was no difference. At both of these ages the animals sed for comparison were males. At subsequent ages the animals sed for comparison were all females. The average for all ages shows hat the relative weight of the pituitary body of the freemartins was 2.0 percent greater than that of the animals used for comparison. 'or the ages at which only females were used for comparison the elative weight of pituitary body was 17.9 percent greater. This hows that the freemartins had a larger number of grams of pituitary ody for each unit of empty-body weight than did the heifers of single irths, although actually the pituitary bodies of the freemartins were maller.

Hall (*29*) studied the pituitary bodies of a number of these free-ıartins and concluded that in cellular structure they were not typical f either the castrated animal or the normal cow, and were intermediate ›etween the two. Evidently this may be taken to indicate a defi-iency of gonadal hormones of either sex.

DEVELOPMENT OF MAMMARY-GLAND TISSUE IN THE FREEMARTIN

Examinations of the form and quantity of mammary-gland tissue n the udders of heifer calves in the breeding herd of the Bureau of)airy Industry at Beltsville, Md., are regularly made by palpation t 2 weeks, and at 1, 2, 3, 4, 5, 6, 9, 12, and 18 months of age. A lescription of the different stages through which the mammary-gland levelopment passes, and a table of expectancy or standard showing ›oth the stage of development and the quantity of tissue found by xtensive studies to represent the average for Holsteins and for Jer-·ys, has been given elsewhere by Swett and Matthews (*62*). When ·aminations are made, the relative degree of advancement in glandu-.r development for the individual calf is evaluated on the basis of the ·andard and is given a grade. Grades range from 1 for the most ·tarded development to 9 for an extremely precocious one. A grade f 5 is given when the mammary-gland development corresponds to he standard or average for the breed. Grades of 4, 3, 2, and 1

represent progressive degrees of retardation, and grades of 6, 7, 8, and 9 indicate progressive degrees of precocity.

It has been the practice to evaluate the mammary-gland tissue development of freemartins as well as normal heifers. Three of the animals listed in table 1 (Nos. 624, 296, and 806) were examined before the system of assigning grades was adopted, and the degree of mammary-gland development was recorded by means of measurements and descriptive terms. Some of the others were evaluated and given grades at one or more ages before the standard was established. The grades shown in table 5 are those given at the time the udder examinations were made.

TABLE 5.—*Grades for mammary-gland development in females of twins of unlike sex*

Animal No.	Grade assigned at age of—									
	2 weeks	1 month	2 months	3 months	4 months	5 months	6 months	9 months	12 months	18 months
691	...	...	2	1	1	1	1	3	3	2
834	5	5	4	1	2	2	2	3	4	4−
1006	...	...	...	4	3	4	5−	5−	4	5
A-70	5	...	5	2	2+	2	3	5+	4+	4−
A-82	6	5	3	1	1	1	1	2	2	...
1209	5	4	3	2−	2+	3	2−	3−	...	...
1421	...	6	4	5−	4−	4	4+	4−	4	...
1427	7	4+	...	3+	3−	2+	4−	...	...	...
1229	4−	5+	4+	3	...	...	...	...	...	...
1212	4−	4	3	2	...	...	...	...	...	...
1485	7	7	4	...	...	...	...	...	...	...
1205 [1]	5	5	8−	...	...	...	...	...	...	...
1408 [1]	6	...	...	...	...	...	...	...	...	...
1200	5	...	...	...	...	...	...	...	...	...
Average grade [2]	5.33	5.00	3.56	2.40	2.25	2.38	2.75	3.57	3.50	3.75
Average (6 animals) [3]	5.25	5.00	3.50	2.00	2.00	2.17	2.17	3.33	3.40	3.33

[1] Post-mortem examination indicated that animal was sexually normal.
[2] Omitting grades for Nos. 1205 and 1408.
[3] Average grade for the 6 animals (691, 834, A-70, A-82, 1209, and 1421) that had grades extending at least from 2 months to 9 months of age, inclusive.

Up to about 1 month of age the mammary development of the freemartin appears to be about the same as that of the normal female. Since there is very little difference in the mammary gland of the bull and the heifer at 1 month of age, it is to be expected that the freemartin would appear to be normal at that age regardless of any abnormality that might be present in the sexual organs. At 2 months the freemartins were, on an average, below the normal in mammary-gland development. The retardation in mammary-gland development became progressively greater until the age of about 4 months. The resulting decline in grade which occurred in almost every individual case does not represent a diminution in quantity of gland tissue but rather a lack of increase which resulted in a greater departure from a progressively increasing normal quantity. After the age of 4 months there was an upward trend in the average grade which, however, did not at any time reach the level that represents normal development. It is possible that an increase in the mass of tissue resulting from fat deposition, rather than an actual increase in glandular tissue, was to some extent responsible for the higher grades being given at the more advanced ages. A grade of 5 was recorded subsequent to 4 months of age in the case of only two animals. Three

animals that died or were slaughtered prior to 4 months of age showed the typical decline in grade.

The two females born twin with bulls (Nos. 1205 and 1408) that were found at time of slaughter to have normal genital development had grades of 5 or above for mammary-gland development. Grades for these two normal heifers are listed, but are not included in the averages in table 5.

A number of the animals listed in table 5 were slaughtered at an early age and consequently had grades covering only the early stages of mammary development. In order to determine whether these individuals affected the decline and subsequent rise in the average grade, an average was determined for the six animals (Nos. 691, 834 A-70, A-82, 1209, and 1421) that had grades extending at least from 2 to 9 months of age, inclusive. There is no marked difference in the two sets of averages (table 5). Moreover, the three animals that were not originally evaluated in terms of grades and those that were graded before the breed standards were definitely established, have been either graded or regraded on the basis of original descriptions measurements, and other data in conformity with current standards in order to determine how much the results in table 5 would be affected. The additions and slight adjustments made did not materially change the results.

Not only were the grades sufficiently low to indicate definite retardation of mammary-gland development in nearly all the freemartins over 3 months of age, but there were several cases in which the type of development did not follow the usual pattern. In such cases the glands were irregular in shape, sometimes indefinite in outline, and in certain instances consisted of a nearly shapeless mass of tissue which did not pass through the normal stages of development from birth to maturity. An abnormal mammary-gland development of this sort is called atypical.

At the time of slaughter the udders were removed. Those from the older freemartins were filled with a formalin solution, frozen, and cut into vertical transverse sections according to the procedure regularly employed by the Bureau in studying the gross anatomy of the udders of cows at the time of slaughter. The mammary glands from the very young animals were dissected in such a manner as to show the form and size of the gland-tissue development in at least two quarters.

The comparative development of the mammary-gland tissue in freemartins and in heifers of similar age that were born singly is illustrated in plates 4 to 14, inclusive. They show the structure of the udder in a transverse, essentially vertical plane passing through one of the rear teats. The photographs were all taken at approximately the same scale.

In selecting the heifers whose udders were used for comparison, an effort was made to obtain animals that were of approximately the same age as the freemartins and that were essentially normal in degree of mammary-gland development. The heifers used for comparison had grades closely approximating the average. Three of the seven heifers used for comparison were younger than the freemartins with which they were compared; two were slightly older; and two were approximately the same age.

Table 6 is presented as an aid to a better understanding of the comparisons made. It shows the identity and the breed of each animal; gives the grade, adjusted to standards now in use, to show the relative degree of mammary-gland development; and indicates the capacity of the secretory system of each udder as determined at the time it was filled with formalin in preparation for freezing and subsequent anatomical studies. Although some of the freemartins had grades as high as 4 for mammary-gland development they were in every case below the normal in this respect. The average of all the freemartin grades shown in table 6 is 3.1 whereas the average for the heifers used for comparison is 5.1. The capacities also were consistently lower for the freemartins than for the heifers used for comparison, except in the case of No. 691 for which there are definite indications of error due to pockets of formalin under the skin. The fact that Holsteins are compared with Jerseys in some instances is of little importance as the mammary-gland development of the two breeds has been shown to differ only slightly, on an average, at most of the ages represented (*62*).

TABLE 6.—*Comparative udder capacity of freemartins and of heifers of similar age that had approximately the average mammary-gland development*

Freemartins					Heifers used for comparison				
Animal No.	Age at death	Breed	Ante-mortem grade for mammary-gland development [1]	Udder capacity	Animal No.	Age at death	Breed	Ante-mortem grade for mammary-gland development [1]	Udder capacity
	Mo. Days			*Pounds*		*Mo. Days*			*Pounds*
691	18 25	Jersey	2	[2] 9.36					
834	18 16	Holstein	4−	4.00	510 U. S.	17 2	Grade Holstein.	6	7.69
1006	18 2	Jersey	4	6.09					
A-70	17 29	Grade Holstein.	4−	6.51					
A-82	14 28	do	[3] 1−	.26	508 U. S.	14 0	do	6−	8.60
1209	12 11	Holstein	[4] 2	.32					
1421	12 6	Jersey	4	3.38	507 U. S.	10 29	do	5+	4.63
806	8 22	Holstein	3−	1.59	68 U. S.	9 2	Holstein	5	4.86
1427	6 1	Jersey	4−	.32	75 U. S.	6 1	do	6	.50
					1259	6 0	do	4	.91
1229	3 0	Holstein	3	.09	686	3 19	Jersey	4	.55

[1] Grades adjusted to conform to current standards.
[2] Capacity appears to be too high. Photograph shows definite indications of collection of formalin between the skin and the mammary gland (pl. 4).
[3] Graded at 14 months 25 days.
[4] Graded at 9 months of age.

Among the four older freemartins (table 6) the udder of No. 691 was relatively small, and only a very small part of the total consisted of gland tissue. Apparently its total size as well as its capacity was exaggerated by the large pocket of formalin which collected under the skin (pl. 4). Fully half of the area of the section of the udder of No. 834 appeared to be fat, the cistern was small and visible ducts were almost lacking (pl. 5). Nearly the same proportion of fat to gland tissue occurred in the section of No. 1006 (pl. 6), but the duct formation was more complete. It did not differ greatly in appearance from the udders of normal heifers of the same age. There was also a small

proportion of gland tissue in the udder of No. A–70, and the gland tissue was not clearly differentiated from the fat which surrounded it (pl. 7). The udder of No. 510 U. S., apparently a normal heifer, was used for comparison with the udders of these four freemartins. Although she was graded slightly above the average in mammary-gland development, she was younger than any of the four freemartins. Her udder was larger than any of the others except possibly that of No. 1006; the gland tissue was fairly widely distributed and clearly differentiated; and the formation of the teat, the cistern, and the duct system appeared to be typical for a heifer of this age (pl. 8).

A striking contrast is shown in the udders of freemartin No. A–82 and of normal heifer No. 508 U. S. The udder of the former was extremely undersized and did not show any clearly differentiated gland-tissue formation. The udder of No. 508 U. S., however, was well developed and generally typical in form, although this heifer was nearly a month younger than No. A–82 (pl. 9, *A* and *B*).

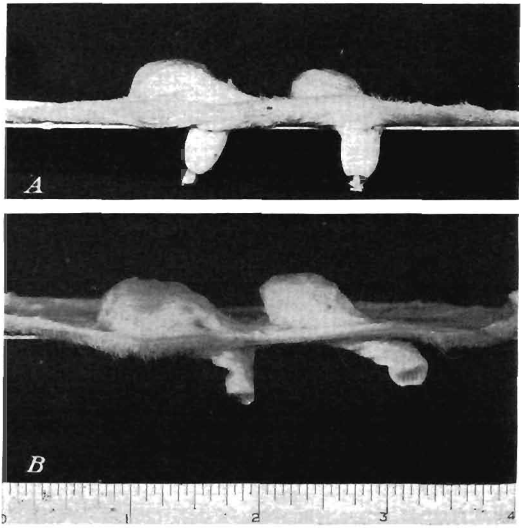
Freemartins No. 1209 and No. 1421 were both more than a month older than normal heifer No. 507 U. S. The mammary development of No. 507 U. S. was approximately normal for her age, as indicated by the grade of 5+. The section from No. 1209 was very small and consisted largely of fat, although the formation of gland tissue was fairly typical (pl. 10, *A*). The gland tissue in the udder of No. 1421 was fairly well distributed but was small in quantity and not clearly differentiated from the fat, and the udder was small (pl. 10, *B*). The section from No. 507 U. S. was much larger than that of either of the freemartins, the gland tissue was widely distributed and more clearly defined, the cistern was of moderate size, and the duct system was fairly well developed (pl. 11).

Freemartin No. 806 was about 10 days younger than normal heifer No. 68 U. S. whose mammary-gland development appeared to be average for heifers of the same age. There was little difference between the two with respect to proportion of gland tissue to fat. The gland tissue was clearly outlined in each, and the duct system appeared to be more fully developed in the freemartin. The freemartin udder, however, was definitely the smaller (pl. 12, *A* and *B*).

Freemartin No. 1427 and normal heifers No. 75 U. S. and No. 1259 were all within 1 day of the same age. The gland tissue in the udder of No. 1427 was not clearly defined and apparently represented only a small proportion of the total area, although the duct system was fairly well developed (pl. 14, *A*). The udders of the two heifers used for comparison were much larger. In No. 75 U. S., the gland tissue represented a large proportion of the total, it was clearly differentiated, and the duct system was unusually well developed (pl. 13, *A*). In No. 1259 the proportion of gland tissue was much lower, it was well differentiated, and the duct system was fairly well developed (pl. 13, *B*). In the case of these two heifers it may be noted that the former was graded 6 and the latter 4 for relative glandular development.

Normal heifer No. 686 was 19 days older than freemartin No. 1229. The udder of the freemartin was slightly the larger but it contained no clearly distinguishable gland formation (pl. 14, *B*), whereas the udder of No. 686 contained a fairly good-sized area of gland tissue with a cistern and some duct formation (pl. 14, *C*).

The dissected mammary glands of No. 1205 and of No. 887 are hown in Figure 1, *A* and *B*. Both were within 3 days of the same ge when slaughtered (2 months, and 2 months 3 days, respectively). No. 1205 was twin born with a bull but was judged normal as a result f post-mortem anatomical findings. Since she was sexually normal, he was not classed as a freemartin and is not listed in table 6. No. 87 was born singly. Apparently she was normal sexually and approx- nately normal in mammary-gland development. The udder devel-



GURE 1.—*A*, Dissection showing the mammary-gland tissue development of No. 1205, a normal heifer born twin with a bull (age 2 months); *B*, a similar dissection of No. 887, a normal heifer born singly (age 2 months, 3 days).

ment of No. 1205 was somewhat more advanced, as determined by lpation, than that of No. 887, although the dissections did not dicate any marked difference.

Most of the freemartin udders illustrated contained a comparatively ge proportion of fat in relation to gland tissue. In many of the emartin udders the gland tissue was not clearly differentiated from fat surrounding it, and in some cases the duct formation appeared arded in development. In most cases the freemartin udders were atively small.

CRITERIA FOR RECOGNIZING THE FREEMARTIN AT AN EARLY AGE

The recognition of an early external manifestation of freemartinism will be helpful to breeders since it may save them the trouble and expense of raising females of mixed twins that at breeding age prove to be sterile. There are a number of characteristics that may indicate freemartinism. Possibly one or two of these will be seen in one individual, and other characteristics may indicate this condition in another individual. Following is a discussion of the characteristics that have appeared to be most indicative of freemartinism in the animals included in this study.

The freemartins on which this report is based were undersized and were inclined to be narrow in relation to height, length, and depth. It is very doubtful, however, whether these characteristics were sufficiently pronounced in these animals to indicate freemartinism. The retarded development of these animals probably was due largely to the fact that they were twins.

A definitely retarded mammary-gland development in a female twin born with a bull may be considered as an indication of freemartinism, although too much stress cannot be placed on it because of the wide variation in degree of udder-tissue development in females of single births. Neither is the external appearance of the udder a reliable indication, since the size and shape of the udder and the length and form of the teats vary greatly in heifers born singly.

Probably more credence may be attached to a retarded mammary-gland development that also fails to pass through the typical stages and occurs in an indefinitely shaped mass of tissue that does not conform to the usual pattern.

Another condition frequently, though not always, associated with freemartinism is an enlarged clitoris. Numerous cases have been observed in which the freemartin had external genitals that appeared to be entirely normal. In other cases the clitoris was so much overdeveloped that it was visible at all times and so obstructed the vulva that the urine spurted upward when the animals urinated (figure 2*A* and *B*). Absence of an enlargement of the clitoris is not necessarily an indication of sexual normality but an enlargement is a very good indication that the animal is a freemartin.

One other abnormality that appears to be quite consistently associated with the freemartin condition is the presence of a fold of skin on the median plane of the body, which varies in size and in length but which sometimes extends from the perineum, a short distance below the vulva, to a point close to the navel. In the region of the udder the fold sometimes hangs down to a marked degree. In one freemartin it resembled a scrotum which occasionally was drawn up until it practically disappeared. Usually the fold of skin contains a cord or bundle of fibers, which may be very pronounced in some individuals. Owing to the location of the fold and the enclosed cord it has been referred to frequently as a "rudimentary penis" (figure 3). The dissection of one of these cords led to the conclusion that it was a modified retractor muscle the function of which, in the bull, is to withdraw the penis into the sheath after erection or protusion. In one other case the so-called cord was sectioned for microscopic study.

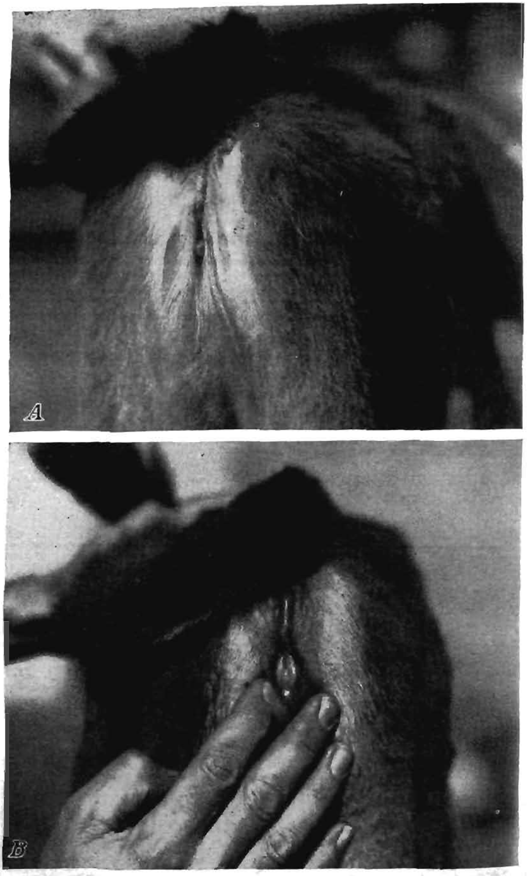


FIGURE 2.—*A*, The protuding clitoris of freemartin No. 691 at 3 months of age; *B*, the less prominent but greatly enlarged clitoris of freemartin No. 1485 at 2 months of age.

It failed to show an organization of cell structure sufficiently definite to identify it as a rudimentary form of any organ or part. Whatever its origin, it was found in a high percentage of the freemartins studied and was absent in the two females twinned with bulls, that were found on post mortem examination to have normal sexual development.

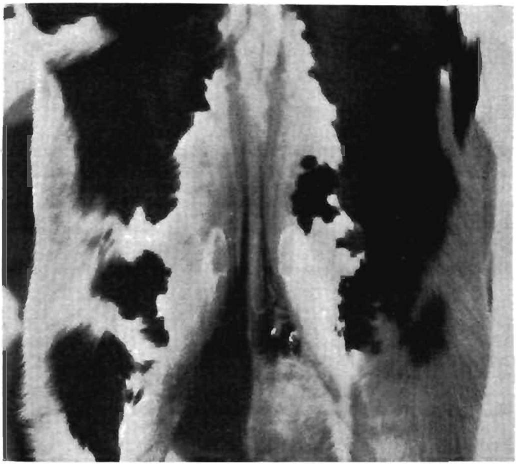


FIGURE 3.—The prominent fold of skin, sometimes enclosing a cord, which has been referred to as a "rudimentary penis."

ABERRATIONS IN ANIMALS STUDIED

Following is a brief description of the physical and anatomical aberration noted in each of the 17 females twinborn with bulls, on which this study is based.

Jersey No. 691 was extremely retarded in mammary-gland development for a period of several months, at no time approaching the breed average. (See table 5.) The glandular development also was abnormal and was an irregular-shaped mass of tissue. At nearly every observation the clitoris was greatly enlarged and the vulva was very small. The presence of the so-called rudimentary penis was very marked at nearly every examination from 3 to 18 months of age, when the animal was slaughtered. At 12 months of age it extended from the clitoris to the navel and at 18 months it was even more pronounced. Post mortem examination showed only a small outline of the uterus, an absence of ovarian tissue, and a vagina (only about 2 inches long) that did not communicate with the rudimentary uterus. The clitoris was about 1.5 inches long, the vulva opening was only

about 0.75 inch in diameter, and there was a strip of "muscle" beneath the skin that extended from the vulva to the umbilicus.

Holstein No. 834 was very much retarded in mammary-gland development from 3 to 12 months but was improved at 18 months of age. The glandular tissue was a very unusual formation with the tissue very indefinite in shape and outline. The clitoris and vulva, however, appeared to be normal. There was a fairly prominent median fold of skin at 3 and 6 months of age but at 18 months the rudimentary penis was relatively small as compared to that of No. 691. Autopsy showed undeveloped genital organs, no trace of uterus except more dense tissue at that place, and only small nodules in the position usually occupied by the ovaries. The vagina was only about 3¼ inches long.

The data available for Jersey No. 624 show that she was extremely retarded in mammary-gland development, and that the glandular formation consisted of a mass of soft, stringy tissue, which lacked definite shape and outline, which was not typical in any respect, and which was one of the most unusual formations yet studied. Only traces of the usual female internal genitalia were found on post-mortem examination.

Jersey No. 1006 was one of the most unusual cases studied. The mammary-gland development of this animal was only slightly retarded; it adhered fairly closely to the usual pattern with regard to the sequence of stages of development; and at 12 months the internal development was not noticeably different from others of similar age except that the gland was slightly undersized. The clitoris showed no signs of enlargement and the vulva was normal. There was, however, a trace of the median fold or rudimentary penis in this animal. She was found on post-mortem examination to be almost devoid of female genital organs, having only a vagina about 3 inches long and two tubelike structures about 1.5 inches long (similar to the horns of a uterus) that seemed to be continued by strands of tissue for about 12 inches. There were small cysts about the size of immature follicles between these strands. A small rudimentary testicle was found on the right side near the usual site of the ovary. On the left side a similar testicle was found, which had descended and lodged on the dorsal surface of the udder. (See pl. 1.)

Grade Holstein No. A-70 was very much retarded in mammary development at 3 to 5 months of age but showed some improvement subsequently. In type of development of mammary-gland tissue the departure from the normal was comparatively slight. The clitoris and vulva were normal. A median fold containing a cord or rudimentary penis which extended from the rear of the udder almost to the navel was noted at 6, 9, 12, and 15 months of age. It was the dissection of the cord in this animal that led to the conclusion it might be retractor penis muscle. She was somewhat more fully developed internally than most of the freemartins studied, the genitals consisting of small ovarylike glands, uterine horns with tubes to vesicula seminalis, and a vagina about 3 inches long, but she had no cervix (pl. 15).

Holstein No. 296 was very much retarded in mammary-gland development, which appeared to deviate sharply from the usual type. At 9 months it was described as the strangest structure yet observed, at 12 months it had very little similarity to the usual type, and before

slaughter it was described as "not at all typical in shape, or development, or in any other way." No reference was made to the clitoris. At 9 months a layer of tissue hung down between the teats the entire length of the udder along the median line and at the time of slaughter also there were indications of a rudimentary penis. Only the most rudimentary development of the internal genital organs was found on post-mortem examination.

Grade Holstein No. A-82 was extremely retarded in mammary-gland development at from 3 to 6 months of age, after which a slight improvement occurred. At 9 months there were indications of some abnormality in type of mammary development, at 12 months the outline was very indistinct, and at 15 months the gland tissue was indistinct in outline and the quarters were not typical in shape or in position. The clitoris was not enlarged. It may even have been slightly smaller than the average. There was only a trace of the cord elsewhere referred to as a rudimentary penis. Autopsy disclosed a 2-inch vagina, and cordlike tissue leading to tubular uterine horns about 1 inch in length, to which were attached shreds of tissue containing very small bodies that may have been ovaries.

Holstein No. 1209 was very much retarded in mammary-gland development from 3 months on. The mammary tissue was slightly but not significantly atypical or abnormal in form. The clitoris was slightly enlarged at nearly every examination. There was no indication of a rudimentary penis except at 9 months, when a trace was noted. Post-mortem examination showed underdeveloped internal genitalia which included a 3-inch vagina, small seminal vesicles, small tubular structures, and small gonadal lumps resembling testicular tissue.

Jersey No. 1421 was only slightly below the breed average in mammary-gland development, which was typical in form, and the clitoris did not show more than a very slight enlargement at any age. The median cord, however, was noted at 3 months and at each examination thereafter. It seemed to increase in size and distinctness and was most noticeable at the rear and along the udder to its front attachment, but not in the region of the navel. Although the external manifestations were less marked than in some of the others it was predicted that this animal would be a freemartin. On autopsy she was found to be devoid of anything approaching normal internal genitalia and to be a typical neuter.

Holstein No. 806 was definitely retarded in mammary-gland development. At 5 and again at 6 months the formation appeared to be somewhat irregular in type, but at 9 months was described as typical but greatly retarded. There were no notations in the record on the condition of the clitoris; but there was a trace of a narrow fold of skin along the median line at 3 months, although no reference was made to a cord formation within it. Her internal genital organs were markedly vestigial and she was undoubtedly a neuter.

Jersey No. 1427 was definitely retarded in mammary-gland development at 3 to 5 months of age but was more nearly normal at 6 months. There were no indications of abnormalities in the form of glandular development. The clitoris, however, showed a marked enlargement. It was so large that it protruded and was exposed all the time, and the ventral extremity of the vulva pointed nearly straight toward the rear.

At first the rudimentary penis was comparatively small and not ve noticeable, but at 6 months it was rather pronounced. Post morte findings showed only rudiments of the usual female internal genitali

Holstein No. 1229 was somewhat retarded in mammary-gland d velopment when slaughtered at 3 months of age. The type of gland lar development at that time was described as indefinite and n comparable to others; not conforming to any stage; a soft, irregul mass of fatty and fibrous tissue. In other words it was an atypic mammary-gland development. The enlargement of the clitoris w only slight. The fold and cord were present but not pronounce Post mortem findings showed that her internal genitals consisted chiel of an undeveloped vagina, two small bulblike objects in the place the uterus, and two small lumps at the usual site of the ovaries.

Holstein No. 1212 was very much retarded in mammary-glan development when examined before slaughter at 3 months of age, b there was no indication of abnormality in type of glandular formatio For this animal the outstanding aberration was the greatly enlarg clitoris. It was about 1 inch long, could be seen without opening t vulva, and occupied a very large part of the vulva, which was in near a horizontal position. The urine spurted upward and outward account of the position of the vulva and the obstruction of the clitor. There was no indication of the co-called rudimentary penis. Po mortem dissectious showed that the vulvalike opening continu forward as a diminutive vagina for about 2 inches and ended in blind pouch. Two testicles with tubes and ampullae were foun The testicles and tubes were in a position similar to that of the ovari and uterus of the normal female.

Jersey No. 1485 had shown better than average mammary-glan development until just before she was slaughtered at the age of months, when it was slightly below the average and definitely abnorm iu form. The clitoris was very pronounced; it was almost full exposed and was clearly visible from a distance, even at the early ag of 16 days. It occupied about one-third of the vulva. There w also a definite indication of a rudimentary penis. At 2 months it co sisted of a very pronounced ridge, clearly visible all the way from t rear attachment of the udder to the navel, and containing a small cor which could be detected at almost any point. It was this animal tha showed the scrotumlike pouch that was occasionally drawn up unt it practically disappeared. Post mortem examination showed tha the genitals consisted of small folliclelike structures at the site of th ovaries, fibrous strands instead of a uterus, and a vagina only abo 1½ iuches long.

Holstein No. 1205 was slaughtered at the age of 2 months. At th time her mammary-gland development was typical in form, and som what advanced iu degree. The clitoris was not enlarged, in fact i appeared to be unusually small. There was no indication of the med an fold or so-called rudimeutary penis or of any of the other indicatio of freemartinism that have been pointed out in the foregoing case Post mortem studies showed that she had all of the organs of a norm female and apparently was normal sexually.

Jersey No. 1408 died at the age of 26 days. Only one examinati had been made (at 14 days). It did not show any abnormality i degree or in type of mammary development. The clitoris was ve

small. No reference was made in the report of the examination to the presence of a rudimentary penis. Autopsy findings indicated that the animal was sexually normal.

Holstein No. 1200 died at the age of 18 days. Only one antimortem examination was made (at 11 days). The glandular development was not notably deficient or unusual in type. The clitoris, however, was very large, very prominent, and visible at all times. It extended above the center of the orifice of the vulva, which stood open above it. There was no indication of the so-called rudimentary penis. The condition of her internal genitals showed that she was undoubtedly an intersexual individual.

Some idea of the extent to which aberrations occur and of their significance may be obtained from table 7. As noted in the discussions, the data for No. 624, No. 296, and No. 806 are incomplete and the absence of a notation does not necessarily indicate that no abnormality occurred. Lack of a notation for any item in the case of the other animals, however, may be considered as a reasonably reliable indication of normalcy for that characteristic.

TABLE 7.—*Physical aberrations noted in 17 females that were twinborn with bulls* [1]

Animal No.	Retarded development of mammary gland	Atypical development of mammary gland	Enlarged clitoris	Presence of rudimentary penis
691	XXX	XX	XXX	XXX
834	XX	XXX	(2)	X
624	XXX	XXX	------	------
1006	X	(3)	(2)	X
A-70	XX	X	(2)	XX
296	XXX	XXX	------	X
A-82	XXX	XX	(2)	X
1209	XXX	X	X	X
1421	X	(2)	X	XX
806	XX	X	------	X
1427	XX	------	XXX	XX
1229	XX	XX	X	X
1212	XXX	------	XXX	None
1485	X	XX	XXX	XXX
1205 [4]	(5)	(2)	(2)	None
1408 [4]	(2)	------	(6)	------
1200	(3)	------	XXX	------

[1] X indicates that the characteristic occurred as a trace or in a slight degree: XX indicates that it occurred in a definitely marked degree; and XXX that it was very marked or extreme.
[2] Normal.
[3] Approximately normal.
[4] Normal internal genitals.
[5] Advanced.
[6] Very small.

A retarded development of the mammary-gland tissue was the most frequently observed abnormality. It was noted in 14 cases. The presence of the median fold of skin between the vulva and the navel, which sometimes enclosed a cord and which was referred to as a rudimentary penis was noted in 12 cases. An atypical form of mammary-gland development was noted in 10 cases and an enlarged clitoris in 8. Some of the freemartins showed definite abnormalities in 1 or 2 characteristics; some in different ones. Others, like Nos. 691, 1209, and 1229 were abnormal in each of the 4 characteristics. Every 1 of the 17 animals showed at least a trace of 1 or more abnormalities except Nos. 1205 and 1408, both of which appeared on post-mortem

examination to be sexually normal. The 15 that showed ante-mortem manifestations of abnormality were all found on post mortem to be deficient in their internal sex equipment.

The contrast in development of internal genitalia between the apparently normal No. 1205 and the freemartin No. 1485 is shown in plate 16, A and B.

SUMMARY AND CONCLUSIONS

For some 2,000 years cattle breeders and investigators have observed that a female calf twinborn with a bull calf is likely to be deficient in sexual development and incapable of reproduction. An animal so born and showing such deficiencies is called a freemartin. Investigators have reported fairly conclusive resnlts indicating that such an individual is genetically a female that has developed from a separate ovum and that the sex modification has resulted from the effects of hormones produced by the gonads of the male twin embryo, which have circulated through the female embryo as a result of fusion of the blood vessels of the two placentas.

Sexual aberrations similar to those found in the freemartin are sometimes observed in other than the bovine species. Sex intergrades resembling the freemartin have been produced experimentally in laboratory animals by injections of sex hormones either into the mother or into the embryo. If freemartinism occurs naturally in connection with multiple births involving both sexes, it appears to be the exception in other species whereas in cattle it is the rule. Authorities differ in opinion as to the frequency of occurrence of normal individuals among heifers that are twinborn with bull calves. An average of the estimates given by such authorities indicates that a normal female may be expected about once in 12 cases of twinning involving both sexes.

This report is based on a study of 17 heifers twin born with bulls. All of those that were kept to breeding age proved to be sexually abnormal and incapable of reproduction. A very wide range in degree of abnormality was found. In some the female internal genitals were modified, in some they were almost lacking, and in some male gonads were found. Only 2 of the 17 were found on post-mortem examination to have normal genital development. As these 2 were 2 months of age or less at the time of death it was, of course, not certain that they would have been capable of reproduction at breeding age despite the apparent normalcy of their genital organs.

Although the data are too limited to warrant definite conclusions, twinning appears to have been habitual in some of the dams of the freemartins studied. Among the dams of the freemartins there was no particular age at which twins of either like or unlike sex seemed most likey to occur.

On an average the freemartins were definitely undersized, especially in live weight. Deficiency in body size seemed to be more pronounced in width than in other body dimensions. Although on an average they were undersized at all of tbe ages studied, there was a slight tendency for the freemartins to approach their respective breed averages more slowly than did heifers twinned with heifers—especially in live weight. The smallness of the freemartins probably was

due to the fact that they were twinborn rather than that they were freemartins. It is very doubtful that either smallness of the individual or any peculiarity in body conformation could be used as a satisfactory basis for distinguishing between freemartins and normal heifers.

On an average the relative size of the organs and body parts, expressed in number of units of weight or measurement per 100 pounds empty body weight, was approximately 10 percent greater for the freemartins than for the animals with which they were compared. Actually the freemartins had the smaller organs. The greater relative size of their organs was due to the fact that the freemartins were more undersized in body weight than in the size of their organs.

The pituitary body was actually smaller in the freemartins than in the animals used for comparison. On the basis of number of grams of pituitary body per unit of empty-body weight, however, the freemartins had the larger pituitary bodies. The freemartin pituitaries that were studied cytologically proved to be intermediate between the condition found in the castrated bovine and that of the normal cow. Presumably this indicates a deficiency in both the male and female sex hormones.

The mammary-gland development of the freemartins as determined by palpation was, on an average, approximately normal up to about 1 month of age after which it was definitely retarded. The greatest retardation occurred at about 4 months after which there appeared to be a tendency for some improvement.

The udders of the freemartins had lower capacities than the udders of normal heifers of the same age, in most cases they contained a high percentage of fat in relation to gland tissue, in many of them the gland tissue was not clearly differentiated from the surrounding fat, and in some the duct formation appeared to be retarded. In most of the freemartins the udders were relatively small.

There are a number of characteristics that appear to be indicative of freemartinism and useful in determining whether or not any given female twin born with a bull calf is likely to be capable of reproduction. Many of the freemartins included in this study were greatly retarded in udder development. In some cases the mammary-gland development was atypical. Another characteristic found in many of the freemartins was an enlarged clitoris; and still another which was very often present was a fold of skin, sometimes containing a cord, which extended along the median plane of the body, part or all of the way from a point above the rear attachment of the udder to the navel. This has been referred to in this study as a "rudimentary penis." In some freemartins all of these characteristics occurred. In others only 1 or 2 could be detected. One or more was found in everyone of the 15 cases studied that proved on autopsy to be deficient in sexual development. None was found in either of the 2 that had normally developed internal genital organs at the time of death.

The chances are slight that the heifer twinned with a bull will be sexually normal. In some cases positive determination probably cannot be made until the animal reaches breeding age. However, if one or more of the abnormalities described are present it probably will be good economy to dispose of the animal.

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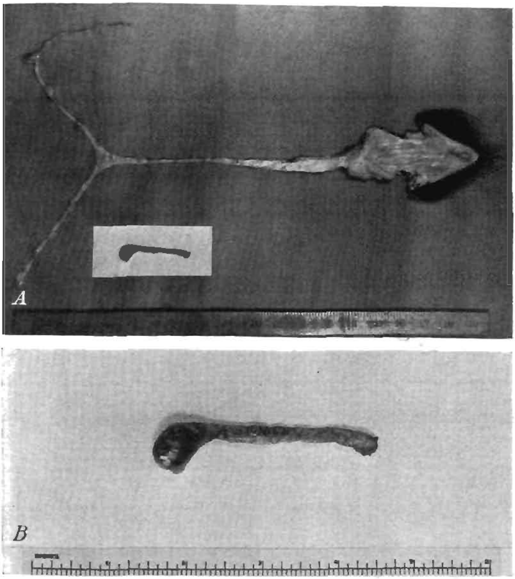
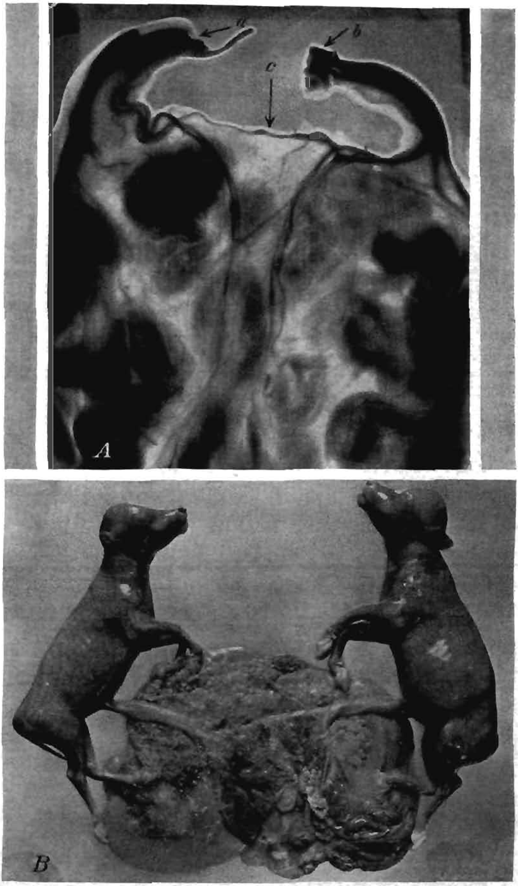


ternal genitals of freemartin No. 1006 at 18 months 2 days of age: *A*, The genital tract: *B*, the descended testicle.

Internal genitals of normal heifer No. 509 U. S. at 16 months of age.

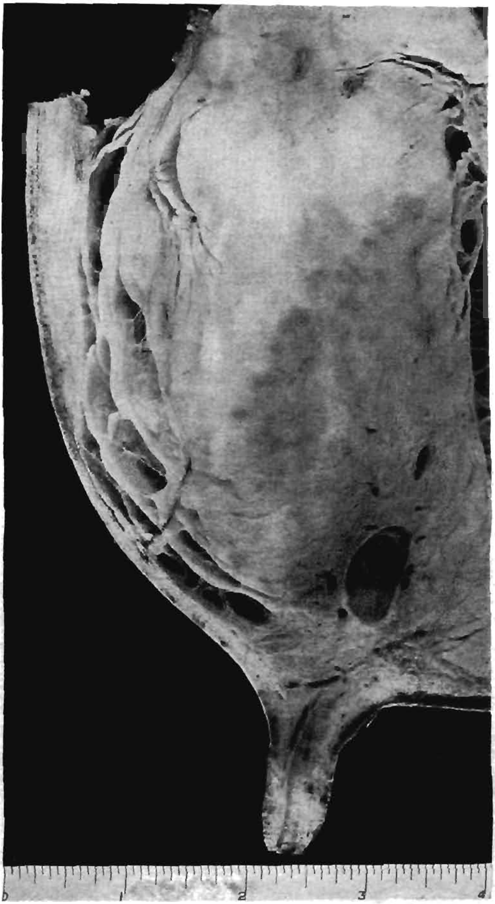


A, Vascular fusion of twin bovine fetuses of cow No. A–40: *a*, Umbilical cord of one fetus; *b*, umbilical cord of the other; *c*, direct vascular connection between them. *B*, Twin bovine fetuses of unlike sex from cow No. 485 showing vascular fusion.

Section through a rear quarter of the udder of freemartin No. 691 at 18 months 25 days of age.

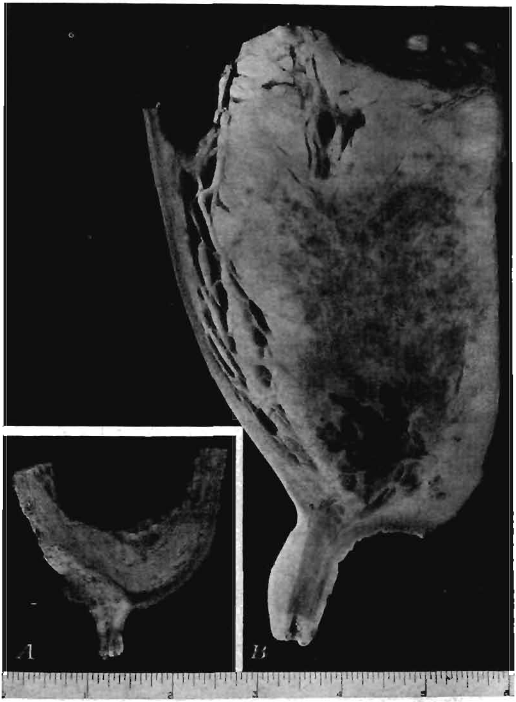
Section through a rear quarter of the udder of freemartin No. 834 at 18 months 16 days of age.

ection through a rear quarter of the udder of freemartin No. 1006 at 18 months 2 days of age.

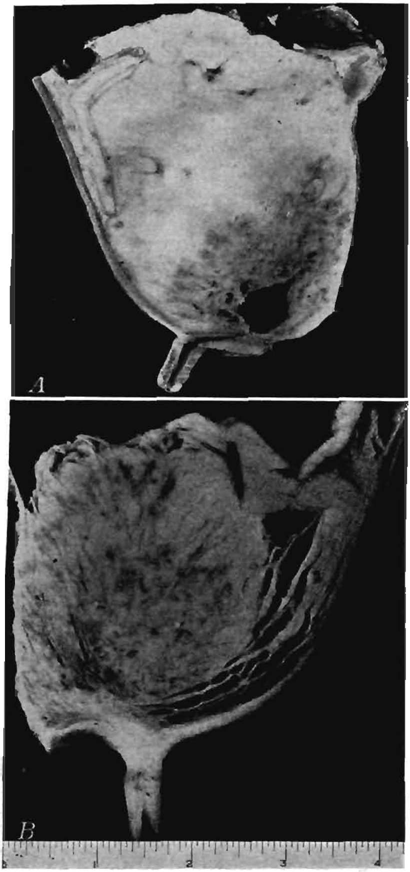


ion through a rear quarter of the udder of freemartin No. A-70 at 17 months 29 days of age.

tion through a rear quarter of the udder of normal heifer No. 510 U. S. at 17 months 2 days of age.

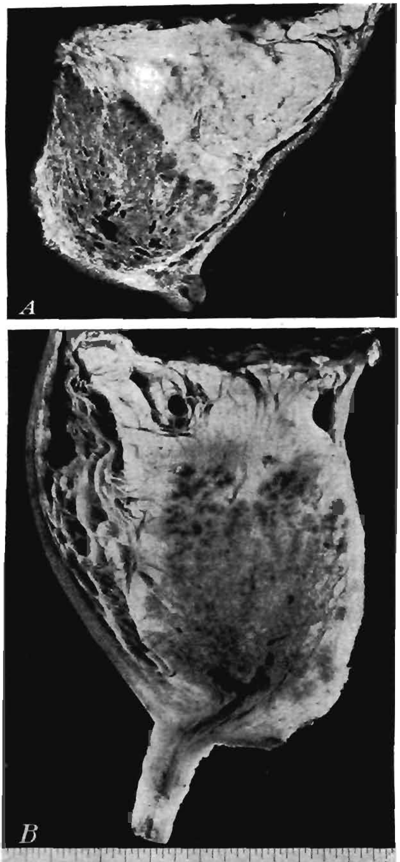


, Section through a rear quarter of the udder of freemartin No. A–82 at 14 months 28 days of age; *B*, section through a rear quarter of the udder of normal heifer No. 508 U. S. at 14 months of age.

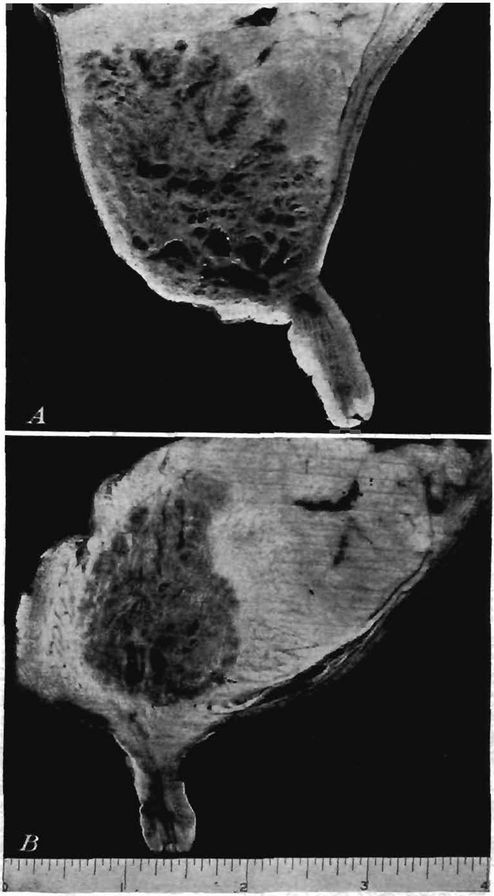


1, Section through a rear quarter of the udder of freemartin No. 1209 at 12 months 11 days of age; *B*, section through a rear quarter of the udder of freemartin No. 1421 at 12 months 6 days of age.

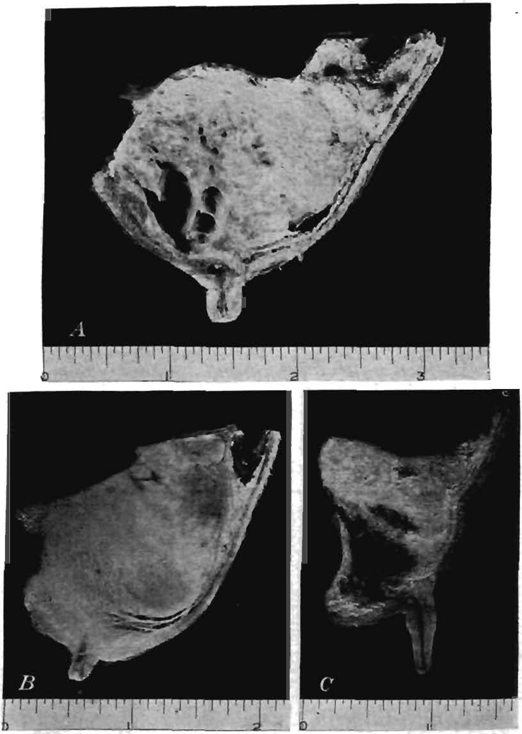
Section through a rear quarter of the udder of normal heifer No. 507 U. S. at 10 months 20 days of age.



A, Section through a rear quarter of the udder of freemartin No. 806 at 8 months 22 days of age; *B*, section through rear quarter of the udder of normal heifer No. 68 U. S. at 9 months 2 days of age.

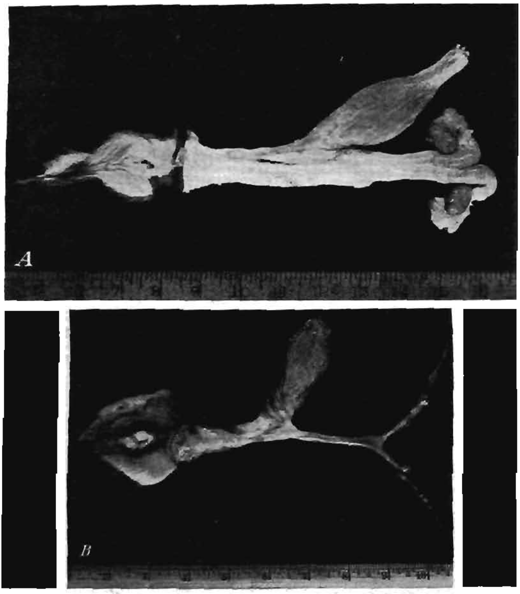


4, Section through a rear quarter of the udder of normal heifer No. 75 U. S. at 6 months 1 day of age; *B*, section through rear quarter of the udder of normal heifer No. 1259 at 6 months of age.



A, Section through a rear quarter of the udder of freemartin No. 1427 at 6 months 1 day of age; *B*, section through a rear quarter of the udder of freemartin No. 1229 at 3 months of age; *C*, section through a rear quarter of the udder of normal heifer No. 686 at 3 months 19 days of age.

Internal genitalia of A-70. This freemartin had reproductive organs of intermediate development, but no cervix.



A, Internal genital tract of No. 1205, twinborn with a bull calf but apparently a normal heifer; *B*, the rudimentary genital tract of freemartin No. 1485 at approximately the same age.

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MOSAIC AND STREAK DISEASES OF ROSE[1]

y PHILIP BRIERLEY, *pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry,* and FLOYD F. SMITH, *entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*[2]

INTRODUCTION

Rose mosaic, or infectious chlorosis, was first described by White *19*)[3] from New Jersey and Massachusetts in 1928. The disease 'as considered as serious and widespread in the United States in ıter reports by White (*1, 20, 21, 22, 23*),[4] Nelson (*13*), and Weiss nd McWhorter,[5] but its importance was discounted by Milbrath *11, 12*). Field evidence suggesting natural spread was advanced y McWhorter.[6] Other chlorotic disorders, particularly in rose nderstocks, were evidently confused with rose mosaic in many of hese early surveys. Some of the early concern over rose mosaic 'as due to claims that affected plants might be killed. Immature Ianetti stocks (*25*) were responsible for outright loss of some plants n the year in which mosaic was first recognized. It is probable that he effects of immaturity were confused with mosaic in some early bservations, since typical rose mosaic is rarely if ever fatal.

The present study was undertaken to determine whether insect 'ectors of rose mosaic occur (1932–35) and to devise means of conrol (1933–38). As the work progressed it became necessary to edefine the symptoms of the disease (*4*), excluding certain types of ymptoms attributed to rose mosaic by others but not found transnissible to hybrid tea roses in the writers' tests. A new estimate of revalence has been made by indexing samples of understocks and y testing transmissibility of chlorotic disorders appearing naturally n roses. Yellow variants of the mosaic pattern were encountered, nd also an apparently undescribed virus disease called "streak" (*3*) ecause of its necrotic effects in certain rose varieties. At present ose mosaic is attracting attention in California, and streak in Texas. A discussion of the relation of nursery practices to spread of these liseases has already appeared (*5*).

[1] Received for publication August 31, 1940. Cooperative investigations by the Division of Fruit and 'egetable Crops and Diseases, Bureau of Plant Industry, and the Division of Truck Crop and Garden nsect Investigations, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

[2] The writers are indebted to a number of nurserymen for supplying samples of commercial understocks, nd to Freeman Weiss, senior pathologist in the Division of Mycology and Disease Survey, Bureau of 'lant Industry, for samples of rose mosaic and a collection of stocks affected with "crinkle" assembled rior to 1933.

[3] Italic numbers in parentheses refer to Literature Cited, p. 659.

[4] WHITE, R. P. AN INFECTIOUS CHLOROSIS OF ROSES. U. S. Dept. Agr. Plant Dis. Rptr. 12: 33–34. 1928. Mimeographed.]

[5] WEISS, FREEMAN, and MCWHORTER, FRANK P. PACIFIC COAST SURVEY FOR ROSE MOSAIC. U. S. Dept. ıgr. Plant Dis. Rptr. 14: 203–205. 1930. [Mimeographed.]

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The writers' type stock of rose mosaic was a plant of Mme. Butterfly and its vegetative offspring. This plant was obtained from a New Jersey greenhouse, and the symptoms were confirmed as typical at the time by White. The stock of healthy Mme. Butterfly came from a greenhouse in Washington, D. C., where it had long been propagated on its own roots. No mosaic was detected in the source stock and no natural infection has appeared in 6 years' experimental culture of the writers' material.

SYMPTOMS

TYPICAL ROSE MOSAIC

The symptoms of typical rose mosaic in the Mme. Butterfly variety are essentially as described by White (*23*), who originally defined the disease. In the course of 6 years' growth under glass and in the field, the writers' type mosaic affected Mme. Butterfly has developed symptoms of several patterns. The typical symptoms are prominent chlorotic areas commonly adjacent to and feathering away from the midrib and accompanied by more or less severe distortion of the leaflet (fig. 1, *A*; also *23*, *fig. 1*, *A*, *B*). These patterns are hereinafter called "typical symptoms." Such chlorotic areas may appear in each leaflet of a leaf, or one or more leaflets may remain symptomless. Not infrequently a whole leaf on an affected shoot may fail to express symptoms, but buds taken from the axils of such symptomless leaves have proved infectious.

A second symptom expressed in the same plant line consists of more or less clearly defined rings, erratic wavy lines or watermark types, and oak-leaf patterns (fig. 1, *D*). White has illustrated this symptom type (*23*, *figs. 2*, *G*; *3*, *C*; *4*, *E*), and Valleau's (*18*, *fig. 26*) rose virus patterns are also of this order. Old leaves that have developed in the greenhouse during the summer months sometimes have shown extensive grayish-yellow areas with green islands (fig. 1, *E*). Cuttings from the type mosaic-affected Mme. Butterfly grown in the field at the Arlington Experiment Farm, Arlington, Va., showed striking yellow vein-banding patterns in May 1934 (fig. 1, *H*). This symptom was expressed only in known mosaic-affected plants and was absent from healthy plants of Mme. Butterfly of the same age that had been grown adjacent to the affected plants.

All the mosaic symptoms in Mme. Butterfly described above have appeared in a single plant line. No evidence of mixture of two or more viruses in this line has been detected in a large number of inoculations to various types of roses and subinoculations back to Mme. Butterfly (see table 5). It is therefore assumed, in the absence of any evidence to the contrary, that the several symptom types mentioned above are responses to infection with one virus, namely, the rose mosaic of White.

The symptoms described for Mme. Butterfly include nearly all the types known to be expressions of typical rose mosaic in the genus *Rosa*. In Ophelia, Radiance, Rapture, and Templar, symptoms are essentially the same as in Mme. Butterfly, the chlorotic patching about the midribs predominating. In Briarcliff, Columbia (fig. 1, *C*), George C. Waud, Odorata (*Rosa odorata* Sweet, P. I. 22994; fig. 1, *B*), Talisman, and The Queen, symptoms are similar but less conspicuously developed. The watermark type and the field expression of prominent yellow

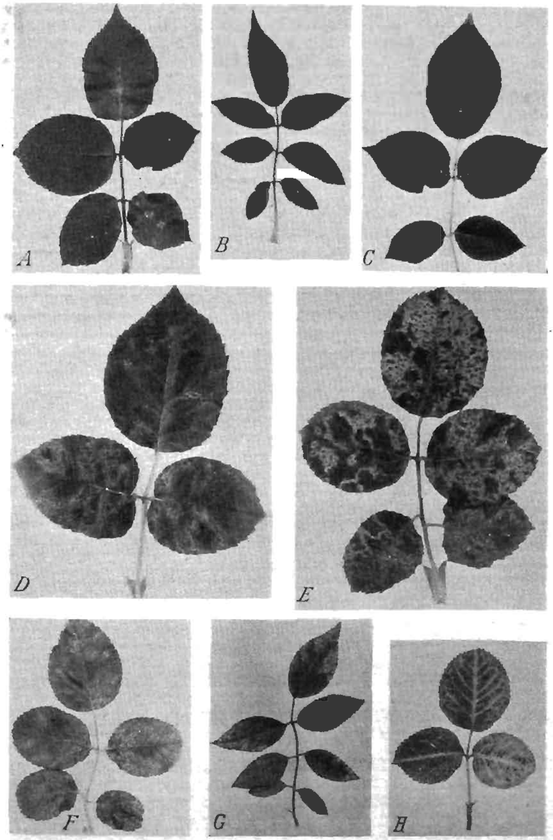


FIGURE 1.—Symptoms of typical rose mosaic. *A*, Characteristic symptoms in Mme. Butterfly; *B*, in Odorata; *C*, in Columbia; *D*, ring and watermark patterns in Mme. Butterfly; *E*, dull-yellow blotching with green islands in Mme. Butterfly; *F*, watermark pattern in Paul's Scarlet Climber; *G*, dull-yellow watermark pattern in field-grown Odorata; *H*, yellow vein banding in field-grown Mme. Butterfly. *C* and *F*, Experimentally produced; others, natural infection.

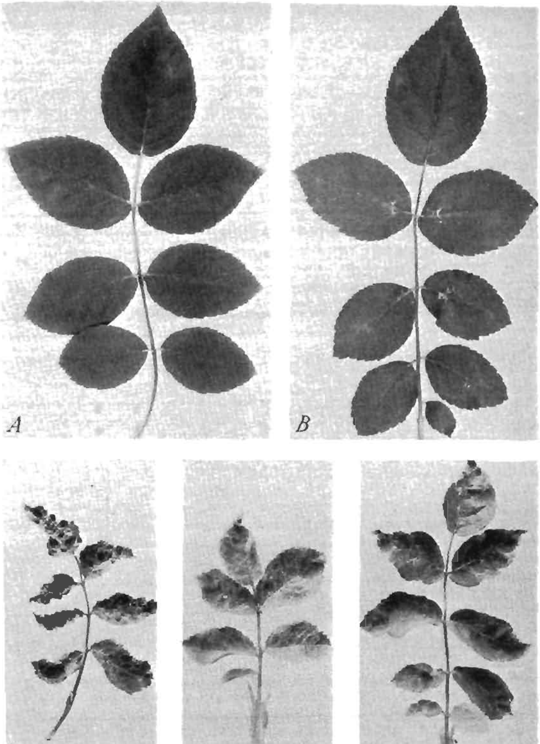
vein banding have appeared in Briarcliff also. In Blaze, Conrad Ferdinand Meyer, *Duchess of Wellington, Joanna Hill, Kaiserin Auguste* Viktoria, Mme. Berthe Fontaine, Mrs. A. R. Waddell, Mrs. John Laing, Paul's Scarlet Climber (fig. 1, *F*), *R. hugonis* Hemsl., *R. nutkana* Presl., *R. wichuraiana* Crép., Souvenir de Claudius Pernet, and Ulrich Brunner, only the oak-leaf-watermark-ring symptoms have been observed. Subinoculation from mosaic-affected plants of the varieties named to healthy Mme. Butterfly always produced symptoms comparable to those in the type plants of affected Mme. Butterfly.

Mosaic has been experimentally produced in the understocks Canina (*Rosa canina* L.), Manetti (a variety of *R. noisettiana* Thory), Multiflora (*R. multiflora* Thunb.), Odorata, Ragged Robin (Gloire des Rosomanes), and Texas Wax (*R. multiflora*×*R. chinensis*). Natural infection has been demonstrated in Manetti, Odorata, and Ragged Robin. In Manetti the symptoms include chlorotic patching about the midrib and also watermark patterns (fig. 2, *B*). Expression of these symptoms is the exception rather than the rule under greenhouse conditions, even in plants proved to be mosaic-affected. For example, in 28 healthy Manetti plants budded to known mosaic hybrid teas during April and May 1934 and grown continuously in the greenhouse, mosaic symptoms were first recognizable in 3 plants on April 9, 1935, nearly 12 months after inoculation. Of the remaining 25 plants, 1 showed mosaic symptoms on June 19, 4 more on July 19, 3 on August 7, and 1 on September 6, 1935. In all, 12 of the 28 plants in this trial showed evidence of mosaic within 16 months after budding. All buds lived and developed into shoots, and all these scion shoots expressed symptoms. In general, the more vigorously growing Manetti plants were more likely to express symptoms. No resistance is believed to be involved here, but merely irregular expression of symptoms. When cuttings were rooted from symptomless plants thus inoculated and buds from healthy Mme. Butterfly were set in these rooted cuttings, they developed into typical mosaic-affected shoots. Similarly, buds from the symptomless Manetti induced mosaic in Mme. Butterfly plants when set in the canes of that variety. Manetti is thus shown capable of masking mosaic under greenhouse conditions.

Diagnosis of mosaic in Manetti in the field is even less trustworthy and is complicated by the frequent occurrence of the crinkle or "rattlesnake" symptoms (fig. 2, *C*, *D*, *E*), which are not transmissible to hybrid tea roses but are more conspicuous than true rose mosaic symptoms in Manetti. White's illustrations of mosaic in Manetti (*23, fig. 1, D, E*) correspond more closely to the crinkle type than to rose mosaic symptoms.

Odorata develops chlorosis and puckering near the midrib or on the larger branch veins (fig. 1, *B*). This symptom is similar to that typically expressed by Briarcliff. The pattern is developed both under glass and in the field with more constancy than are symptoms in Manetti. Watermark patterns may also occur (*23, fig. 2, G*). In the field a dull-yellow watermark has appeared (fig. 1, *G*) in mosaic-affected Odorata.

Rosa multiflora develops ring patterns or watermark types, the prevailing pattern varying with the strain of this understock. Both the Japonica and the Chenault strains of *R. multiflora* have expressed symptoms regularly in the greenhouse, and diagnosis from symptoms



[F]IGURE 2.—Mosaic and crinkle in Manetti. *A*, Healthy specimen; *B*, mosaic-affected specimen from greenhouse, showing faint watermark patterns as well as typical mosaic symptoms; *C*, *D*, *E*, crinkle or "rattlesnake" patterns in Oregon field-grown Manetti. Natural infections. (Photograph by F. P. McWhorter.)

(see fig. 8, *A;* also *23, fig. 2, B, E*) has not appeared consistently ir Multiflora plants budded to mosaic, and when appearing naturally has not proved transmissible to Mme. Butterfly.

*Ragged Robin, when experimentally infected, showed inconspicu*ous watermark patterns in a few leaves and occasional weak expres*sions of typical symptoms. The chlorotic speckle pattern common i*r Ragged Robin has not proved transmissible to Mme. Butterfly. Th*e weak expression of mosaic symptoms in this variety and the prevalenc*e of a distinct and apparently innocuous speckle present problems o diagnosis closely comparable to those in Manetti.

Texas Wax developed occasional well-defined watermark pattern after bud inoculation. Experimentally infected plants, when trans ferred to the field, showed a dull-yellow watermark similar to tha expressed by Odorata. Marked crinkling and dwarfing of leaflets o vigorous young canes, common in Texas Wax in the vicinity o Washington, has not been found transmissible to hybrid tea roses.

The type symptoms of rose mosaic remain clearly defined in leave of Mme. Butterfly and Odorata that were pressed 5 years ago. Like wise oak-leaf patterns in C. F. Meyer and George C. Waud are readily recognizable after a like interval.

YELLOW MOSAICS[7]

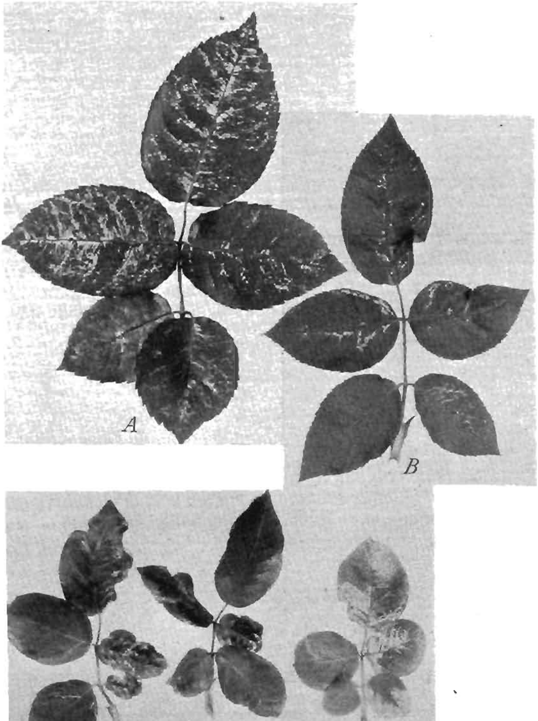
Five collections of mosaic from naturally affected roses differec from the typical rose mosaic in symptoms. The sources of the yellow types were Talisman from Rockville, Md.; C. F. Meyer from New York, N. Y.; Margaret McGredy from West Grove, Pa.; and Irish Charm and an unnamed hybrid perpetual rose from gardens at the Arlington Experiment Farm, Arlington, Va. The Rockville collection was *from a greenhouse; all others were from the field. In each instance a single* affected plant was found among many plants free from mosaic symp*toms. The symptoms expressed in the original affected plants and o*n transfer to Mme. Butterfly differ from the typical rose mosaic chiefly *in color.* The chlorotic areas are in general a brighter and lighter yellow than in typical mosaic[8] and are often extensively developed *and very conspicuous. In most varieties and most strains there i*s less tendency to puckering of the leaves in the yellow types, but one *of the Arlington farm collections may produce marked puckering.*

Comparison of the five yellow mosaic collections in Briarcliff, Mme. *Butterfly, C. F. Meyer,* Margaret McGredy, *and* Talisman *over a* period of a year or more showed differences among the yellow types. No two of the five yellow variants were alike *in symptom expression* in all five varieties. The observed differences were of the order commonly used in distinguishing strains of a virus. The Talisman strain induced bright conspicuous symptoms in most varieties (fig. 3, *A*, *B*), and was distinct from typical rose mosaic in all varieties except Paul's Scarlet Climber. In Briarcliff it often produced bright-yellow blotching in young canes. In one Multiflora seedling it produced tan streaks and patches in the bark of young canes, dwarfing of both

[7] *Thomas and Massey (17) distinguish rose mosaics 1, 2, and 3. Their rose mosaic 1 is evidently White's* rose mosaic (the writers' "typical rose mosaic"). Their rose mosaics 2 and 3 are similar to those the writers have termed "yellow mosaics."

[8] L. C. C. Krieger made the following matched-color readings in Maerz and Paul (*10*). Yellow mosaic (Talisman strain) in Briarcliff leaves: M. and P. 10K2 (specimen not quite as bright); normal leaf abou M. and P. 23L8. Typical mosaic in Briarcliff leaves: M. and P. 22 between K and L6; normal leaf M. and P. 23L7, a little greener. Typical mosaic in Mme. Butterfly leaf: M. and P. 22L6; normal leaf M. and P 23L between 7 and 8. Dull-yellow pattern of typical mosaic in old leaves of Mme. Butterfly: M. and P.

leaves and shoots, and dieback, in addition to yellow watermark patterns. Tan cane lesions developed in *Rosa nutkana* also. The C. F.



IGURE 3.—Symptoms of yellow mosaics: *A*, Talisman strain in Mme. Butterfly; *B*, Talisman strain in Briarcliff; *C*, one of two Arlington farm strains in the source plant, an unnamed hybrid perpetual variety. *A* and *B*, Experimentally produced infections; specimens from greenhouse; *C*, natural infections, specimens from field.

leyer strain expressed distinctive virus patterns in Briarcliff, C. F. leyer, Mme. Butterfly, and Margaret McGredy. The Irish Charm

strain developed watermark patterns in C. F. Meyer, Margaret McGredy, and Talisman, but no distinct evidence of infection in the other two test varieties. In the source plant it developed prominent yellow blotching and ring markings without distortion of the leaflets.

The Margaret McGredy strain was recognizable as a yellow variant of rose mosaic in all test varieties. The strain from an unnamed hybrid perpetual rose from the Arlington farm (fig. 3, *C*) gave strikingly conspicuous symptoms in the source plant. In the test varieties it was hardly recognizable as a virus disease except for inconspicuous watermarking in Briarcliff and necrotic effects in Talisman. In Talisman it behaved much like rose streak, involving necrosis of the bud followed by general dieback and death of the plant. Failure to produce streak effects in Briarcliff and Mme. Butterfly distinguishes the strain from the streak disease, however. This test was repeated three times, and in each of the four trials Talisman died, with similar symptoms.

Differentiation of strains in the rose mosaic group is of no particular importance and there is no assurance that they are strains of one virus. The tendency of some well-defined yellow mosaics to revert to crinkle symptoms in certain varieties is, however, interesting. In view of this behavior it would not be surprising to find that some of the crinkle symptoms in hybrid perpetuals (see fig. 8, *B*) represent transmissible types, although the writers have not been able to demonstrate it. Evidently Briarcliff and Mme. Butterfly are not particularly responsive as test varieties for possible viruses of the crinkle type.

White's "albication of Golden Ophelia" appears from his illustration (*23, fig. 3, E*) to be a characteristic yellow mosaic. Milbrath's "albication of Souvenir de Claudius Pernet" (*12, fig. 126*) also appears to belong to this group, showing distinct oak-leaf margins between the yellowed and green leaf areas. The writers have been unable to get material of either of these earlier reported yellow mosaics for comparison.

ROSE STREAK

Rose streak disease, which previously has been described briefly (*2*), was first noticed at the Arlington farm in the late summer of 1933. It has since been found at Beltsville, Md., Washington, D. C., and New York, N. Y. Characteristic symptoms were noted at the Arlington farm in October 1934, in 60 varieties of rose, including teas, hybrid teas, hybrid perpetuals, hybrid multifloras, hybrid wichuraianas, hybrid rugosas, hybrid Bengals, Noisettes, Chinas, and polyanthas. Specimens submitted from Texas by J. J. Taubenhaus and E. W. Lyle showed typical streak symptoms. Subinoculation from Lyle's specimens of the Edel variety, showing ring symptoms in both canes and leaves, produced the characteristic symptoms of this disease in Mme. Butterfly.

Symptoms of three classes have proved reliable for diagnosis of streak: (1) Brownish rings (fig. 4, *A*) and brown vein banding (fig. 4, *B*) in fully expanded leaves, usually accompanied by brownish or greenish, often water-soaked, ring patterns in canes (fig. 5) (the cane symptoms have been reproduced in experimentally produced streak infections in Odorata, Multiflora, and Silver Moon under glass, but they are less conspicuous and the coloring is more dilute than in the field); (2) green senescence designs similar to the brown patterns (fig. 4, *B*, *C*), which are often expressed in leaves that are later pre-

maturely abcised; and (3) a yellowish-green vein banding (fig. 4, *D*) in certain hybrid multiflora and hybrid wichuraiana roses. This symptom is usually accompanied by greenish water-soaked rings or dull-

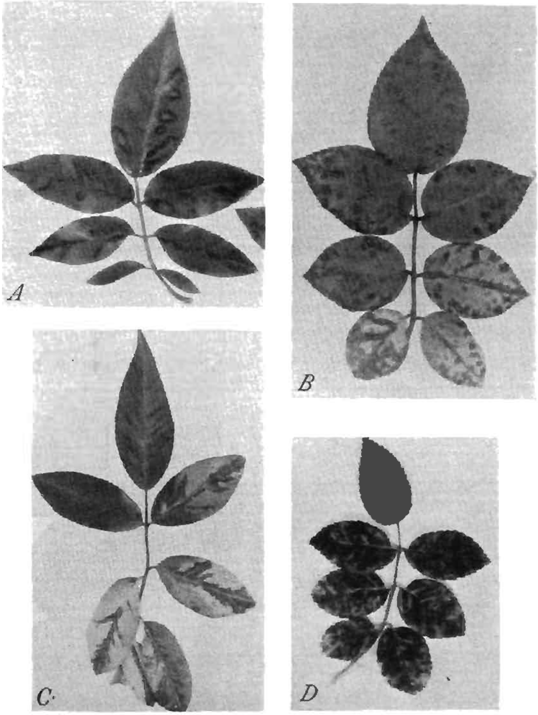


FIGURE 4.—Leaf symptoms of rose streak: *A*, Brown rings in Odorata; *B*, discontinuous brown vein banding and senescence patterns in Van Fleet Hybrid No. 36; *C*, senescence patterns in Odorata; *D*, yellowish-green vein banding in Jean Girin. All natural infections; specimens from the field.

brownish rings in the canes (fig. 5). White's figures of "veinal chlorosis" (*23*, *fig. 5*, *B*, *C*, *D*) strongly resemble this streak symptom, but White did not report cane symptoms. A plant of the hybrid rugosa rose Sarah Van Fleet, showing a bright-yellow mottling in

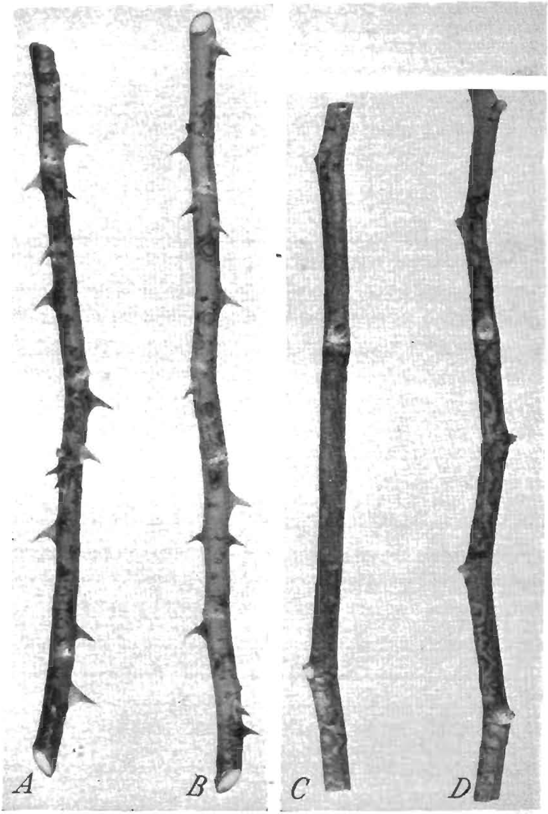


FIGURE 5.—Symptoms of rose streak disease in rose canes: A and B, Canes of Jean Girin; C and D, canes of Miss G. Mesman. All natural infections; specimens from the field, Arlington, Va.

leaves but no cane lesions, yielded only streak on subinoculation to Mme. Butterfly. The rugosa group seems the most difficult of the common garden types in which to diagnose streak, but some varieties show well-defined cane symptoms when affected.

In addition to these streak symptoms found in affected roses in nature, an experimentally produced symptom deserves mention. When streak-affected buds are set in canes of certain hybrid tea roses, such as Briarcliff, Mme. Butterfly, Ophelia, and others, the stock turns nearly black [9] and necrotic (fig. 6) about the inserted bud soon after union has been established.

Sometimes nearly black, necrotic secondary lesions appear on young lateral branches arising below the inserted bud (fig. 7, *A*). Commonly

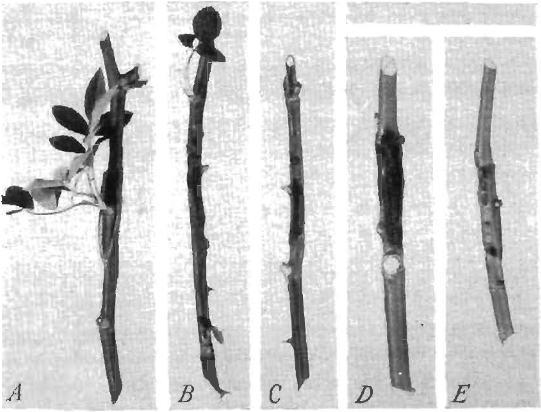


FIGURE 6.—Primary necrotic lesions of rose streak in Mme. Butterfly and Briarcliff. *A, B, C,* Mme. Butterfly canes budded with Odorata November 6, 1934: Odorata is healthy in *A*, streak-affected in *B* and *C*. *D, E,* Briarcliff: *D*, Budded with streak-affected Miss G. Mesman October 5, 1934; *E*, budded with streak-affected Silver Moon December 5, 1934. Photographed January 31, 1935.

the stem is girdled at the site of the bud, and the distal parts die and the leaves wither but persist (fig. 7, *B*). This is a necrotic primary lesion in the cane, and the virus is capable of limited further movement under favorable conditions. Such effects have not been recognized in nature, but if natural spread of the streak virus were taking place, comparable but perhaps smaller primary lesions of this type should appear in varieties of this group.

[9] L. C. C. Krieger made the following matched-color readings based on Maerz and Paul (*10*) and on Ridgway (*14*). Streak in Mme. Butterfly cane. M. and P. 56L1 (specimen very much darker); Ridgway 13M, maroon (specimen very much darker). Streak in Multiflora canes, M. and P. 56L1 (specimen darker); Ridgway 13M, maroon but with more black in it. Streak in *R. wichuraiana* canes, M. and P. 6L10 to a rifle lighter upwards on the cane; Ridgway 15K, morocco red (specimen a little lighter).

FIGURE 7.—Symptoms of rose streak in Mme. Butterfly (6-inch pots): *A*, Budded from streak-affected American Pillar July 15, 1934. Note necrosis about the bud and also four secondary necrotic lesions on cane at left. The leaf at base was shed from top of left cane. *B*, Budded from streak-affected Multiflora September 2, 1933. The Multiflora bud has been cut away from cane at right; the lesion has advanced downward, involving the lateral at right, on which withered leaves persist. Cane at left remains unaffected. Photographed August 30, 1934.

VIRUSLIKE PATTERNS NOT FOUND TO BE TRANSMISSIBLE

Various crinkle types, often accompanied by dwarfing and twisting of leaflets and whole leaves, have proved nontransmissible to hybrid teas in trials by the writers. Most important is the crinkle type in Manetti, known in the Northwest as "rattlesnake" (fig. 2, *C*, *D*, *E*). Mild symptoms consist of yellowish-green speckling without distortion or dwarfing of the leaves; the spots are small and angular and may be very densely distributed without consistent association with the veinlets. A more severe expression involves crinkling and distortion of the leaflets, especially at the tips. The most striking expression of this series of symptoms includes marked dwarfing of the leaflet, puckering throughout, and frequently twisting of the petiole.

Odorata and Texas Wax often display conspicuous dwarfing, puckering, and twisting of leaves in zones a foot or more in length on vigorous canes. Although chlorotic expressions are less evident in these stocks, the symptoms mentioned appear under similar conditions and seem to belong to the same series as the Manetti symptoms described. A misalinement of leaflets without dwarfing is a milder expression commonly appearing at either end of a zone of severe symptoms. In Multiflora the faulty alinement of leaflets on petioles and twisting of petioles also occur under conditions that permit development of "rattlesnake" in Manetti and its counterparts in Odorata and Texas Wax. Chlorosis and dwarfing do not commonly accompany this twisting in Multiflora.

Attempts have been made to correlate the crinkle type in Manetti, Texas Wax, and Odorata with environmental factors. In the spring of 1934 Manetti from two sources was brought at intervals from cold storage into a cold propagating house. Crinkle or "rattlesnake" was very prevalent in March, but plants moved to a warmer greenhouse developed normal new foliage. Later plantings of similar stock showed milder crinkle in the propagating house. A lot of Manetti, removed from cold storage on March 23, developed normally in a warm greenhouse but expressed some crinkle in a cool house and in an outdoor frame. Field expression of the crinkle type has shown a marked tendency to show zonation characterized by series of crinkled leaves alternating with series of normal leaves on the same cane. This suggests a controlling environmental factor that fluctuates periodically, such as temperature, light intensity, or moisture. However, no clear relation with such environmental factors was detected in a preliminary study of the development of zonation in field Manetti.

Manetti, Texas Wax, and Odorata stocks all showed a marked tendency to develop crinkle when grown under cheesecloth shade on low land at the Arlington farm. Manetti of Oregon, California, and English origin showed crinkle August 14, 1934, 6 weeks after being moved to this site. Texas Wax, Odorata, and Odorata seedlings similarly expressed crinkle in the new growth produced after establishment on this shaded site. However, cloth houses on a hillside at Beltsville, Md., failed to induce typical crinkle. Shade is evidently not the sole determining factor in the expression of this symptom.

Interest in the factors that determine the expression of crinkle declined with the accumulation of evidence that this symptom is unrelated to rose mosaic. Healthy Mme. Butterfly budded into the Manetti stocks mentioned here and into other lots with crinkle history developed into mosaic-free plants. Crinkle might be the expression

of a virus disease that the writers have failed to detect because the test plants used (chiefly Briarcliff and Mme. Butterfly) were not suited to its expression. If such is the case, the expression of symptoms in Manetti is very sensitive to environment, and the virus is exceedingly widespread in rose stocks. Until some evidence of virus etiology is advanced, the writers prefer to interpret the trouble as a direct environmental effect. The original interest in crinkle or

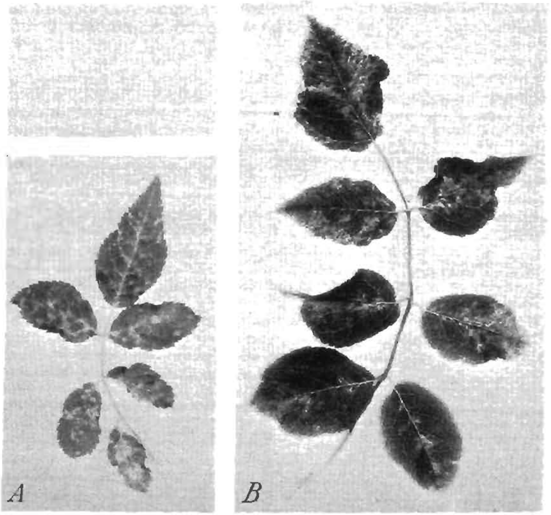


FIGURE 8.—Symptoms suggestive of virus diseases but not found transmissible to hybrid tea roses: *A*, "Speckle" type in Multiflora from greenhouse; *B*, mosaiclike pattern in unnamed hybrid perpetual rose from field.

"rattlesnake" is attributable to three factors: (1) The symptoms suggest virus etiology; (2) investigators observed crinkle appearing in Manetti as a sequence to mosaic inoculation, or observed mosaic appearing in roses worked on crinkled stocks; and (3) surveys, conducted in advance of adequate research, revealed that crinkle was common where mosaic was believed to be common. The second of these factors may now be explained. Since in Manetti crinkle is more common and more easily diagnosed than mosaic, one would expect mosaic to occur occasionally in crinkled Manetti, but no high correlation should appear between crinkle and mosaic content. Again, if mosaic is budded into Manetti the true symptoms (fig. 2, *B*) are usually inconspicuous and irregularly expressed, but crinkle

patterns (fig. 2, *C*, *D*, *E*) may appear whenever the environmental conditions are suitable.

The commercial importance of crinkle types in rose understocks depends on the effect of this peculiarity on their performance as understocks. If crinkled Manetti is as well suited as symptomless Manetti for use as a stock for hybrid tea roses, then crinkle is of technical interest only. No critical test of this point has been made, but growers' trials have shown no conspicuous difference in performance of crinkled as compared with symptomless Manetti. The writers' experience has brought to light no adverse effects in hybrid tea roses worked on stocks with crinkle history, but a carefully designed experiment would be necessary to determine whether there is any detectable difference in merit as stocks between crinkled and normal-appearing Manetti.

A speckle type in Multiflora has appeared sporadically in the field and under glass in the writers' material (fig. 8, *A*). Affected leaves are thickly sprinkled with angular chlorotic spots of varying size. The leaflets are often dwarfed but do not show the characteristic puckering associated with crinkle or "rattlesnake." Speckle is typically expressed under glass, but "rattlesnake" is largely suppressed and only the milder phases become evident under the writers' greenhouse conditions. Thirty-seven of 329 seedlings raised from speckled Multiflora parents showed speckle in turn. This symptom appeared in seedlings grown continuously under glass as well as in those grown in the open. Ragged Robin may also develop a similar chlorotic spotting under glass and in the field.

TABLE 1.—*Rose species and varieties showing symptoms suggestive of virus disease but producing no mosaic symptoms in Briarcliff or Mme. Butterfly when budded into one or both varieties*

Source plant	Source locality	Symptoms in source plant
Briarcliff	Arlington, Va.	Speckle.
Do	Rockville, Md.	Do.
Dr. Eckener	West Grove, Pa.	Do.
Julia Countess D'Ortig	Vineland, Ont.	Crinkle.
Mme. Berthe Fontaine	Arlington, Va.	Do.
Manetti	do	Do.
Do	Doylestown, Pa.	Do.
Do	Hillsboro, Oreg.	Crinkle (fig. 2, *C*, *D*, *E*).
Mrs. F. R. Pierson	Arlington, Va.	Speckle, striped flower.
Mrs. John Laing	Vineland, Ont.	Crinkle.
Odorata	Arlington, Va.	Do.
Do	do	Albication.
Ragged Robin	do	Speckle.
Rosa alberti Reg.	Beltsville, Md.	Crinkle.
R. spinosissima altaica Rehd.	do	Do.
R. canina L.	Arlington, Va.	Albication.
R. multiflora Thunb.	do	Crinkle.
Do	do	Speckle (fig. 8, *A*).
R. nutkana Presl.	Hillsboro, Oreg.	Do.
R. pisocarpa A. Gr.	Beltsville, Md.	Crinkle.
R. rugosa Thunb.	Madison, Wis.	Yellow mottle.
R. rugosa Thunb. var. *heterophylla* Hort.	West Grove, Pa.	Crinkle.
R. villosa L.	Beltsville, Md.	Yellow crinkle.
R. webbiana Wall.	do	Yellow mottle.
R. willmottiae Hemsl.	West Grove, Pa.	Speckle, vein clearing.
R. xanthina Lindl.	New York, N. Y.	Speckle.
Striped La France	Arlington, Va.	Crinkle.
Texas Wax	do	Do.
The Queen	Madison, N. J.	Speckle.
Ulrich Brunner	Ithaca, N. Y.	Do.
White Maman Cochet	Arlington, Va.	Crinkle.
York and Lancaster	West Grove, Pa.	Speckle, striped flower.
Unnamed	Beltsville, Md.	Crinkle.
Do	do	Vein clearing.
Unnamed (6 varieties)	Arlington, Va.	Crinkle (fig. 8, *B*).

Speckle and crinkle symptoms occur in hybrid tea and hybrid perpetual roses in the field and less commonly and less prominently in the greenhouse. A number of rose species and varieties showing such symptoms are listed in table 1. None of the source types listed there have induced distinctive symptoms in healthy Briarcliff or Mme. Butterfly. Although this is certainly not conclusive evidence that these symptoms do not represent virus diseases, it is convincing proof that they are not symptoms of rose mosaic. As suggested in the section on yellow mosaics, some of the crinkle patterns in hybrid perpetuals may prove to be virus diseases.

EXPRESSION OF SYMPTOMS IN THE FIELD

In 1934 roses of 5 varieties, experimentally infected with mosaic in the greenhouse, were grown in the field at the Arlington farm to determine the reliability of symptom expression in known mosaic-affected material. The plants were examined at intervals for recognizable mosaic symptoms. During the season 12 of 20 Briarcliff plants, 27 of 27 Mme. Butterfly, 3 of 4 Canina, and 5 of 5 Ophelia expressed symptoms sufficiently distinct for reliable diagnosis, and one mosaic-affected Duchess of Wellington failed to do so. In the same season 4 of 5 mosaic-affected Canina in a cloth house developed distinctive oak-leaf and watermark patterns.

In 1935, a similar experiment was set up at Beltsville, Md., and observed through 1936 and in part through 1937. The observations on expression of rose mosaic are summarized in table 2. The number of plants observed in this experiment changed as individuals died and as additional plants were set from time to time. The number showing symptoms varied as new leaves developed and old leaves were lost. Thus a plant might show symptoms at one reading and none at the next.

In a parallel trial the Talisman strain of yellow mosaic expressed recognizable symptoms in single plants of Briarcliff, Canina, Joanna Hill, Mme. Butterfly, Multiflora, Odorata, and Texas Wax with great regularity; in Manetti symptoms were recognizable on two of the five dates of observation; in Ragged Robin symptoms were not recognized. In a further parallel trial, single plants inoculated with buds from streak-affected Silver Moon were observed through three seasons in the field. Texas Wax expressed streak symptoms at three dates out of five; Multiflora showed symptoms once; Canina, Joanna Hil Manetti, and Ragged Robin remained symptomless throughout th period of observation.

It is clear from these records that symptom expression is uncertai in plants known to be infected with mosaic. Symptoms of the Talis man yellow strain are more regularly expressed, but still may be lackin in Manetti and Ragged Robin. Streak expression is erratic even i Texas Wax and Multiflora, while no expression of symptoms wa detected in Canina and Manetti, which are susceptible to systemi invasion. The performance of Joanna Hill and Ragged Robin ha less bearing on the question of symptom expression since these varieties have not been shown to be susceptible to streak. Diagnosis of the diseases in these trials was hampered by slow growth of the plants during dry weather and by loss of leaves from leaf diseases at times Such conditions may be encountered in commercial rose culture also.

and the trials are thus probably representative of what can be expected in the diagnosis of mosaic and streak in rose nurseries.

TABLE 2.—*Symptom expression of rose mosaic in the field at Beltsville, Md., in plants experimentally infected when set in March 1935, or later*

Variety	June 7, 1935		Aug. 21, 1935		Oct. 12, 1935		Sept. 9, 1936		Oct. 30, 1937	
	Plants observed	Plants affected	Plants observed	Plants affected	Plants observed	Plants affected	Plants observed	Plants affected	Plants observed	Plants affected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
riarcliff	3	1	2	1	3	3	2	2		
'anina	3	3	3	2	3	2	3	3	3	1
oanna Hill	1	0	3	0	3	0	2	1		
Ime. Butterfly	3	3	3	2	3	3	2	2		
Ianetti	3	0	3	2	3	0	3	1	3	0
Iultiflora	1	0	2	1	2	2	2	1	2	2
'dorata	3	0	3	3	3	3	3	0		
.agged Robin	1	1	1	1	1	1	1	1		
'exas Wax	3	1	3	3	3	0	3	3	3	0

DISTRIBUTION AND PREVALENCE

Naturally occurring rose mosaic from 20 sources has been confirmed y transfer to healthy Mme. Butterfly. These sources include 17 arieties of roses and rose stocks (Blaze, C. F. Meyer, Duchess of Vellington, Georg Arends, George C. Waud, Irish Charm, Joanna Iill, Laurent Carle, Mme. Butterfly, Manetti, Margaret McGredy, Irs. F. R. Pierson, Odorata, Ragged Robin, Talisman, Templar, and ne unnamed). Seven States (New York, New Jersey, Pennsylania, Maryland, Virginia, Oregon, California) and the Province of ntario were represented. Five of the 20 collections were yellow ypes, which consistently differed in symptoms from the typical iosaic; the remaining 15 sources were indistinguishable from the type n transfer to Mme. Butterfly. This evidence supports the general iew that rose mosaic is widespread. Because of the systemic nature the disease and the general shipment of roses and rose understocks, osaic may be expected to appear sporadically throughout this ountry and elsewhere. The writers' samples are believed to be presentative of rose mosaic in the United States and Canada, and cept for the yellow variants there is no evidence that the type of osaic varies from one section to another.

Previous survey records of the prevalence of rose mosaic are now en to question, since crinkle and speckle symptoms were previously cepted as characteristic of rose mosaic. In the field the more common nderstocks, Manetti, Multiflora, Odorata, Ragged Robin, and Texas ax, all commonly express symptoms which suggest virus patterns id which were formerly accepted as evidence of rose infection. or example, in a collection of supposed mosaic-affected stocks sembled at the Arlington farm prior to 1933, indexing by means Briarcliff and Mme. Butterfly buds revealed 1 mosaic-affected ant in 89 Manetti, 1 in 21 Odorata, and no mosaic present in 27 ultiflora or in 14 Texas Wax plants (table 3). It was therefore ought desirable to index samples of commercial understocks to termine the percentage of typical rose mosaic naturally occurring. dexing was carried out either by rooting the cuttings under test and dding them to healthy Mme. Butterfly (fig. 9) or budding from the

FIGURE 9.—Healthy Mme. Butterfly budded on Manetti to test for presence of mosaic in this stock: *A* is affected with mosaic; *B* and *C* are normal. Budded June 4, 1934, in 6-inch pots. Photographed August 30, 1934.

test stocks into canes of healthy Mme. Butterfly. Index tests were kept under observation for several months, or longer than was required for expression of symptoms following transfer of known mosaic-affected buds under comparable conditions. The period necessary for expression of symptoms varied with the growth rate of the plants under test and with other factors.

The results of indexing understocks are shown in table 3. No mosaic was found in 103 Texas Wax and 27 Multiflora plants. One of 21 plants of Odorata proved to be affected, and 3 Ragged Robin plants of 208 had mosaic. Two large lots of Manetti proved to be 2.9 and 12.8 percent affected, and two others were less than 1 percent affected. In addition to the Manetti tabulated (table 3), 1 of 55 Joanna Hill plants and 5 of 31 Templar plants, received from a California nursery as "dormant buds" in Manetti, showed mosaic when these buds expanded. No mosaic was found in samples of the Shafter, Calif., stock of 1933 after it had grown for 2 years at Babylon, N. Y., Hillsboro, Oreg., or Portland, Oreg. No evidence appeared from these tests to indicate that typical rose mosaic spreads in the field in Oregon or New York in the absence of budding and grafting operations. The prevalence of mosaic in understocks is somewhat lower than has been claimed, but still high enough to permit extensive spread by indiscriminate budding. The yellow mosaic types have been found as isolated plants only, as previously mentioned. No yellow types were found in the understocks indexed.

TABLE 3.—*Prevalence of rose mosaic in understocks as determined by budding to the Mme. Butterfly variety*

Stock	Source	Source year	Plants indexed	Plants showing mosaic
			Number	*Number*
Manetti	England	1929	17	0
Do	do	1930	24	0
Do	Hillsboro, Oreg	1930	14	1
Do	do	1931	8	0
Do	do	1933	202	1
Do	Portland, Oreg	1930	2	0
Do	do	1931	16	0
Do	Irvington, Calif	1935	86	11
Do	Montebello, Calif	1935	105	3
Do	Shafter, Calif	1932	28	0
Do	do	1933	156	1
Do	Shafter, 1933; Babylon, N. Y. (2 years)	1935	36	0
Do	Shafter, 1933; Hillsboro, Oreg. (2 years)	1935	63	0
Do	Shafter, 1933; Portland, Oreg. (2 years)	1935	47	0
Do	Miscellaneous		8	0
Multiflora	Texas	1930	7	0
Do	Arlington, Va	1931	20	0
Odorata	Hillsboro, Oreg	1930	6	0
Do	Burlingame, Calif	1930	6	1
Do	Sumner, Wash	1930	2	0
Do	Arlington, Va	1931	7	0
Ragged Robin	Hemet, Calif	1935	86	1
Do	Montebello, Calif	1930	3	0
Do	Ontario, Calif	1935	74	1
Do	Puente, Calif	1935	41	1
Do	Miscellaneous	1931	4	0
Texas Wax	Scottsville, Tex	1930	14	0
Do	do	1934	89	0

Streak of roses is limited in known distribution to Maryland, New York, Texas, Virginia, and the District of Columbia. The virus from some 30 sources has reacted uniformly, showing no evidence of differentiation into strains. This disease has never appeared in the com-

mercial understocks indexed, but has been demonstrated in Odorata and Multiflora growing at the Arlington farm. Streak was prevalent at the Arlington farm in a collection of rose species and varieties later discarded. Thirty percent infection was recognizable in one collection of varieties said to have been acquired over a period of years from many nurseries. However, the possibility of increase by propagation on affected stocks cannot be entirely excluded. These circumstances suggested natural spread of the disease at the Arlington farm, but spread has not been proved. At Beltsville, Md., 80 healthy Silver Moon plants failed to show any symptoms of streak after 4 years' growth in the midst of a collection of 1,200 roses that included a considerable number of streak-affected plants, some of which were in contact with Silver Moon test plants. Present evidence, therefore, indicates that no natural spread of streak occurs, but the possibility remains that spread may occur in certain seasons or in other localities.

TRANSMISSIBILITY

TRANSMISSION BY BUDDING AND GRAFTING

Typical rose mosaic, yellow mosaics, and streak are readily transmitted to susceptible roses by various methods of budding and grafting, but not by juice inoculation insofar as is known. Inarch grafts, patch grafts, splice buds, and T-buds have all proved effective when union was established. The last-named method has been used most extensively because of its convenience.

The interval between budding and the appearance of the first recognizable symptoms of these diseases is highly variable. During this period union must be accomplished, and the virus must increase and move to a growing point in which young leaves are expanding. Canes are of approximately the same stage of growth when suitable for budding, but the growth of the inserted bud and shifts in shoot dominance after budding are subject to wide variations. Apparently the best circumstances for prompt production of symptoms include the rapid development of a young lateral shoot close below the transferred bud.

The shortest incubation periods recorded by the writers for rose mosaic are 20 days for 1 transfer from Mme. Butterfly to Mme. Butterfly, 22 days once, 26 days 3 times, and a large number of records of 28 to 49 days with other variety combinations. Under unfavorable circumstances, symptoms have been delayed for 12 months or more.

For yellow mosaics the minimum observed period was 17 days for a transfer from C. F. Meyer to Mme. Butterfly, with records of 26 days, 30 days, and many of 40 days for other combinations. Some yellow mosaic transfers were first evident after periods of 6 and 7 months under less favorable conditions.

For streak the shortest recorded period was 18 days in a transfer from Van Fleet No. 46 to Briarcliff. Periods of 27 days were recorded a number of times, and many of 40 days or less. A few instances of delayed expression of streak were observed also. There is no evidence here of important differences that might be helpful in distinguishing or separating the viruses. All appear to have about the same minimum incubation period in a given variety, and in all the period is subject to wide variation according to variety, growth, food movement, and shifts of shoot dominance in the rose used as a test plant.

None of the three types of disease is transmitted if the diseased bud dies or is removed before union with the test plant has taken place. In a series of 18 Briarcliff plants inoculated by budding to Talisman affected with the Talisman strain of yellow mosaic, no transmission resulted in 3 plants from which the inserted mosaic buds were removed after 3 days' contact, and none in 3 plants in which the buds died without forming a union during 16 to 25 days' contact. Twelve plants from which the affected buds were removed after 8 to 25 days all became infected with mosaic. No evidence of union was visible at the end of 4 days. After 8 days cell division was recognizable, and after 12 days the buds were beginning to adhere to the stock. Symptoms appeared 1 month to 5½ months after budding.

In a similar experiment, 12 healthy Mme. Butterfly plants were budded with streak-affected Silver Moon buds, which were removed after intervals of 5, 10, and 15 days or left in place. Streak failed to develop in 3 plants from which the infected buds were removed after 5 days. The 9 plants on which streak-affected buds were left in contact for 10 days or more all developed typical streak lesions in 3 months or less. Cell division and adhesion of the bud to the stock were evident after 10 days' contact. After 6½ months all streak lesions were removed by cuts 4½ inches below the bud scars. No further streak symptoms developed thereafter.

All attempts to transmit mosaic and streak by needle and rubbing methods have failed, but these trials have hardly been sufficiently extensive to be conclusive. No natural infection of mosaic or streak has appeared in the writers' collection of roses in 5 years' observation, although knives and pruning shears have been used on diseased and healthy roses without precautions against contamination.

Seedlings from roses affected with mosaic or streak have never carried these diseases in the writers' trials. Twelve seedlings of *Rosa wichuraiana*, 1 of Silver Moon, 27 of Odorata, and 123 of Multiflora were grown from streak-affected parents, but showed no symptoms of the disease. All the parent plants except Odorata were proved to be affected by budding trials; the Odorata parent was diagnosed as streak-affected from symptoms only. Healthy Silver Moon was budded into 9 of the Multiflora seedlings as a further test for the presence of the streak virus in these seedlings; these Silver Moon buds grew into normal, healthy plants. Small populations of seedlings have been grown from mosaic-affected plants of Mme. Butterfly, but no mosaic has appeared in these. It is highly improbable that either mosaic or streak is seed-borne in roses.

The Chenault strain of Multiflora is said to be the progeny, both by seed and by cuttings, of a single plant still growing at the United States Horticultural Station at Beltsville, Md. The parent plant and its vegetative progeny carry streak, as shown by symptoms and by bud transfer to Mme. Butterfly, Odorata, and Silver Moon; but seedlings from this plant have been uniformly free in the writers' experience. The presence of the streak virus in this importation, and in other introduced specimens of *Rosa multiflora* and *R. saturata* Bak., might suggest the possibility that the virus was imported in these species; but they are said to have been brought in as seeds rather than as cuttings. Again, distribution of affected Multiflora cuttings or of Van Fleet roses worked on such rooted cuttings is a possible factor in the distribution of streak in this country. Regardless of whether these possibilities

represent actual truths, it is obviously wise to rely on seed propagation of understocks unless they are known to be virus-free.

Confirmation of streak infection in a variegated variety of *Rosa wichuraiana* from the Arlington farm made possible a striking demonstration of the contrasting behavior of a genetic variegation and a virus disease above and below a graft union. The variegated and streak-affected *R. wichuraiana*, established as a scion shoot after budding into healthy Odorata, showed both the genetic and the virus patterns above the union, but only the streak was transmitted to the stock. This material is unusually well suited for demonstrating this standard test for transmissibility.

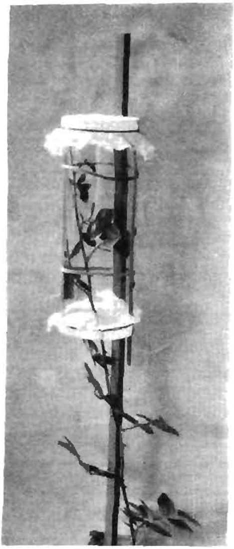


FIGURE 10.—Cage used in insect transmission experiments: The barrel of the cage is of celluloid; the ends are covered with cheesecloth, and the lower end is sewed to make a tight seal, without wrinkles, about the rose cane.

TRANSMISSION EXPERIMENTS WITH INSECTS

Investigations to determine what insects might be capable of transmitting the mosaic and streak diseases of roses were largely exploratory. An effort was made to test all potential vectors, chiefly Homoptera, that occurred on roses or nearby vegetation. Such insect species were obtained by frequent collections in plantings of roses at the Arlington farm, from 1932 to 1935. Collections were also made on survey trips to nurseries and greenhouses in Maryland, Pennsylvania, New Jersey, New York, Ohio, and the District of Columbia.

The number of insects belonging to species capable of breeding on roses was increased by rearing individuals in laboratory cages. Insects belonging to other species died within a short time if confined to rose; therefore tests with such insects were restricted to collected material. All individuals used in experimental tests were preserved and identified by specialists.[10]

In testing for insect transmission, collected or reared insects were confined for 24 hours to several days on tender growths of rose plants infected with either mosaic or streak; they were then transferred to actively growing shoots of healthy plants, where they were permitted to feed until they died, or else for a maximum period of 1 month. A celluloid cage was used to confine insects on terminals of any length

[10] A. C. Mason, Harold Morrison, and P. W. Oman, Division of Insect Identification, Bureau of Entomology and Plant Quarantine.

and was held at any desired height by rubber bands against a stake thrust into the pot of soil (fig. 10). The bottom of the cage was closed by a piece of cheesecloth cut to the center, sewed in place around the stem and thence to the margin, and held in place by rubber bands placed over the branch before putting on the cage. A suction apparatus was employed for introducing or removing insects from the cages, except that aphids were introduced on leaves and removed with a brush. In the case of potted plants all transfers were performed within a transfer chamber to prevent the escape of insects. Most of the experiments were made on potted plants in the greenhouse, but some were conducted on field-grown plants in cheesecloth cages. After all surviving insects had been killed by spraying with pyrethrum extract and the cages had been removed, the plants were maintained in an actively growing condition for periods ranging from 8 to 26 months, so that the development of any disease symptoms might be observed.

The insect species and numbers of individuals used in 229 transmission tests with rose mosaic and in 152 tests with streak are given in table 4. No symptoms indicating transmission of either disease developed in any of the exposed plants. The insects tested included 42 species in the families Cicadellidae, Cercopidae, Membracidae, Araeopidae, Fulgoridae, Aphiidae, and Coccidae, in the order Homoptera, and one species of thrips (order Thysanoptera). As indicated in table 4, a number of species were not tested for transmission of both diseases because these occurred in very small numbers in the areas from which collections were made. It was not considered practicable to rear such less common species in large numbers on their particular, favored hosts for more extensive testing, since it became increasingly evident that natural spread of the two diseases did not occur in the region concerned. The experiments did include, however, all species commonly found on roses in the field or greenhouse and several species that are known vectors of other virus diseases.

In addition to the tests as given in table 4, the following tests were made in an effort to transmit by means of their known vectors recognized viruses from other hosts to the rose variety Mme. Butterfly. In 3 tests, each with 4 adults of *Philaenus leucophthalmus* (L.), strawberry crinkle was not transmitted to rose. In 4 tests with 100 individuals of *Capitophorus fragaefolii* (Ckll.), crinkle was transmitted from strawberry to strawberry, whereas no symptoms developed on 3 rose plants exposed to 75 aphids in parallel tests. In 3 tests with 54 aphids, *Amphorophora rubi* (Kalt.), the vector did not transmit red raspberry mosaic from infected Latham red raspberries to rose; nor was aster yellows transmitted from China aster to 6 rose plants by 75 viruliferous adults of *Macrosteles divisus* (Uhler).

TABLE 4.—*Summary of tests of insects for capacity to transmit the virus of ro[illegible] mosaic or of rose streak, 1932 to 1935, Arlington, Va.*[1]

Insect tested	Mosaic		Streak	
	Plants exposed	Insects in test	Plants exposed	Insects in test
	Number	*Number*	*Number*	*Number*
Family Cicadellidae:				
Aceratagallia sanguinolenta Prov	2	15	8	[illegible]
Agallia constricta Van D.	1	12	10	1
Balclutha impicta (Van D.)	2	2	-	-
Carneocephala flaviceps Riley	1	12	5	[illegible]
Chlorotettix galbanatus Van D	-	-	1	
Chlorotettix viridius Van D	7	11	5	[illegible]
Deltocephalus flavicostus Stål	-	-	2	
Draeculacephala mollipes (Say)	16	95	6	[illegible]
Empoasca fabae (Harris)	23	136	6	[illegible]
Empoasca maligna (Walsh)	7	110	-	-
Erythroneura sp	8	15	-	-
Exitianus obscurinervis Stål	2	36	3	
Graphocephala coccinea (Forst.)	2	23	3	
Graphocephala versuta (Say)	-	-	9	[illegible]
Gypona octolineata var. *striata* Burm	5	37	2	[illegible]
Idiocerus fitchi Van D	1	10	-	-
Kolla bifida (Say)	1	15	-	-
Macrosteles divisus (Uhler)	7	74	4	
Norvellina seminuda (Say)	2	2	1	
Oncometopia lateralis (F.)	6	38	4	2
Phlepsis irroratus (Say)	5	32	9	3
Polyamia inimica (Say)	1	16	-	-
Polyamia obtecta (O. and B.)	-	-	2	
Polyamia weedi (Van D.)	-	-	1	
Scaphytopius acutus (Say)	9	60	10	7
Scaphytopius frontalis (Van D.)	1	13	1	1
Stirellus bicolor (Van D.)	-	-	2	
Stirellus obtutus Van D	-	-	1	
Thamnotettix nigrifrons (Forbes)	5	59	8	5
Typhlocyba pomaria McA	7	33	2	2
Family Cercopidae:				
Lepyronia quadrangularis (Say)	-	-	1	
Philaenus leucophthalmus (L.)	48	116	7	[illegible]
Family Membracidae:				
Ceresa bubalus (F.)	1	1	-	-
Family Araeopidae:				
Delphacodes puella (Van D.)	-	-	1	
Liburniella ornata Stål	-	-	4	1
Family Fulgoridae:				
Scolops sulcipes (Say)	-	-	1	
Family Aphiidae:				
Capitophorus fragaefolii (Ckll.)	3	105	1	2
Macrosiphum rosae (L.)	6	86	9	25
Macrosiphum solanifolii (Ashm.)	32	519	15	25
Myzus porosus Sanderson	13	274	4	12
Family Coccidae:				
Phenacoccus gossypii T. and C.	4	38	-	-
Pseudococcus citri Risso	4	82	-	-
Order Thysanoptera:				
Frankliniella tritici (Fitch)	-	-	4	4[illegible]

[1] The source of virus was Mme. Butterfly for rose mosaic, Silver Moon for rose streak; healthby Mme Butterfly served as test plants. No transmission occurred in any test.

The habits of some of the insect species employed in the above-described transmission tests and the injury they cause on rose ar[illegible] described in a separate publication *(15)*.

VARIETAL REACTIONS

All roses adequately tested have been found susceptible to typical rose mosaic (table 5). The characteristic symptom pattern and the severity of the effects vary, but no variety has been found immune. The subinoculations listed in table 5 were made not only to confirm transmission as evidenced by symptom expression but to determine whether the virus could be recovered unchanged. As explained in the discussion of symptoms, several distinct patterns have been ex-

pressed in the type collection of mosaic-affected Mme. Butterfly including typical rose mosaic, oak-leaf and watermark patterns, dull yellow rings, and broad yellow vein banding. Duchess of Wellingto and Joanna Hill when naturally affected have shown only the oak leaf-watermark symptoms. When experimentally infected wit mosaic from the type in Mme. Butterfly, these varieties and also C. F Meyer, Kaiserin Auguste Viktoria, Manetti, Paul's Scarlet Climbe Ragged Robin, Silver Moon, Souvenir de Claudius Pernet, Texa Wax, Canina, *Rosa hugonis*, and Ulrich Brunner have developed onl or principally these same oak-leaf and watermark designs. On retur inoculations to healthy Mme. Butterfly, however, the virus induce the typical symptoms as well as the watermark patterns, and unde suitable conditions the dull-yellow rings also. Budding from oak-lea Duchess of Wellington has induced typical symptoms as well as wate mark patterns in Mme. Butterfly in each of five trials and in Opheli in two trials. Similarly, typical symptoms and watermark hav developed in Mme. Butterfly following bud transfer from Souvenir d Claudius Pernet showing sparse and mild watermark patterns in tw trials, and in single inoculations from Blaze, George C. Waud, Joann Hill, Kaiserin Auguste Viktoria, and Texas Wax.

TABLE 5.—*Results of inoculation of rose species and varieties with typical ro mosaic, yellow mosaic (Talisman strain), and streak*

[Affected buds were inserted in the varieties tested, and subinoculations were made as indicated]

Species or variety inoculated	Rose mosaic		Yellow mosaic		Rose streak	
	Symptoms produced	Subinoculations	Symptoms produced	Subinoculations	Symptoms produced	Subinoculation
Briarcliff	+	+	+	+	+	−
Columbia	+	+	+	+	+	
Conrad Ferdinand Meyer	+		+	+	−	
Duchess of Wellington	+	+	+		+	+
Gardenia	+				+	+
Joanna Hill	+	+	+		−	
Kaiserin Auguste Viktoria	+	+	+	+	+	+
Mme. Berthe Fontaine	+					
Mme. Butterfly	+	+	+	+	+	−
Manetti	+	+	+	+	−	+
Margaret McGredy	+		+	+	−	
Mrs. Arthur Robert Waddell	+					
Odorata	+	+	+	+	+	+
Ophelia	+	+	+	+	+	
Paul's Scarlet Climber	+	+	+			
Radiance	+		+		+	
Ragged Robin	+	+	+		−	
Rapture	+	+	+		+	
Silver Moon	+	+	+		+	+
Souvenir de Claudius Pernet	+	+	+	+	−	−
Talisman	+		+	+	+	
Templar	+	+	+		+	−
Texas Wax	+	+	+		+	+
The Queen	+					
Rosa canina	+	+	+		+	+
Rosa hugonis	+				−	−
Rosa multiflora	+	+	+	+	+	+
Rosa nutkana	+	+	+		+	+
Rosa wichuraiana	+		+		+	+
Ulrich Brunner	+		+		−	

The milder typical symptoms characeristic of mosaic in Briarcli as well as watermark patterns, have been induced in this variety al in a number of inoculations by budding from roses showing or oak-leaf or watermark symptoms. It is apparent that the mild a

inconspicuous symptoms developed in such varieties represent merely varietal responses to typical rose mosaic, since the more conspicuous symptoms can be reproduced from them on transfer to a suitable variety. Varieties reacting like Mme. Butterfly are most severely injured by mosaic. In these the distortion and chlorosis of leaflets are usually conspicuous enough to affect the salability of the cut flowers. Shortening of internodes or general dwarfing of affected plants has not been observed in the writers' pot cultures. The flowers of mosaic-affected plants have not commonly proved defective in the writers' material, although such effects are reported by White [11] and by Nelson (*13*) to occur under conditions of commercial rose culture. It has not been practicable in the writers' work to lay out a critical experiment on comparative performance of healthy and mosaic-affected roses under forcing conditions. In a trial conducted by White (*24*)[12] mosaic-affected plants of the Talisman variety were little if at all inferior to healthy Talisman plants in number of salable flowers or in stem length but a larger proportion of defective blooms were cut from the mosaic-affected plants.

Talisman, Briarcliff, and others of the group developing mosaic symptoms milder than those of Mme. Butterfly, but otherwise similar in pattern, are evidently less injured than Mme. Butterfly. Others, such as Joanna Hill and Duchess of Wellington, which develop symptoms of the ring or watermark type when affected, are so little injured that the disease would be recognized only on close scrutiny.

In the yellow mosaic group the Talisman strain, which alone has been tested extensively, develops prominent leaf symptoms in a larger number of rose varieties than does typical rose mosaic. Cut flowers of affected plants would tend to be of lower sale value because of these conspicuous leaf patterns. Like typical rose mosaic, this yellow type has affected all roses adequately tested.

In the streak disease of roses sharp differences in varietal reaction appear. One group of varieties is subject to systemic infection by the streak virus: e. g., American Pillar, Canina, Clio, Duchess of Wellington, Gardenia, Jean Girin, Kaiserin Auguste Viktoria, Kitchner of Khartoum, Multiflora, Newport Fairy, Odorata, *Rosa nutkana, R. wichuraiana*, Sarah Van Fleet, Silver Moon, Texas Wax, Veilchenblau. Such varieties, when affected, display one or more of the characteristic symptoms described above, and any bud suitable for transfer will convey the virus to a susceptible healthy variety. Manetti is evidently allied to this group, inasmuch as systemic infection has been proved by subinoculation, but no characteristic streak symptoms were detected in this understock variety in the writers' trials. Streak has likewise been recovered from symptomless plants of Canina and Multiflora that were slow to express symptoms after inoculation, from Sarah Van Fleet, and from two unnamed roses of the Rugosa type that were naturally infected but symptomless at the time. However, it would be premature to create a subgroup for varieties subject to systemic invasion by the streak virus but showing no symptoms, since symptom expression is highly variable in varieties in which the disease is best known.

A second group of roses is subject to necrotic local lesions only: e. g. Briarcliff and Mme. Butterfly (fig. 6), Columbia, Ophelia, Radiance.

[11] See footnote 4.

[12] NEW JERSEY AGRICULTURAL EXPERIMENT STATION. ROSE MOSAIC NOTES. N. J. Agr. Expt. Sta. Nursery Dis. Notes 5 (11): 2-4. 1933. [Mimeographed.]

Rapture, and Templar. The virus may move a short distance into the stock and induce secondary necrotic lesions in young canes near the inserted streak-affected bud (fig. 7, *A*). After a few weeks or months all affected tissue is dead and the lesions cease to enlarge (fig. 7, *B*).

Subinoculation from any bud suitable for transfer fails to carry the virus to a healthy susceptible test plant. As indicated in table 5, all subinoculations from Briarcliff (five attempts) and Mme. Butterfly (seven attempts) showing the characteristic local cane lesions of streak have failed to recover the virus. It is assumed that a similar result would be obtained from Columbia, Ophelia, Radiance, Rapture, and Templar since these varieties are uniform with the two tested in response to this virus. Two series of subinoculations were attempted with a view to determining how far the streak virus advanced beyond its recognizable symptoms. In one series buds were taken from Mme. Butterfly 1½ and 4 inches above and ½, 8, and 15 inches below a streak-affected Silver Moon bud 3 months after the latter was set and at a time when the necrotic lesion was still enlarging about the Silver Moon bud. None of these buds conveyed streak to healthy Mme. Butterfly into which they were set. The failure to recover streak from buds ½ inch below and 1½ inches above an active primary necrotic cane lesion indicates that the streak virus is closely confined to the area of visible symptoms in varieties showing this response.

The inserted streak-affected bud is killed by the girdling lesion and the cane above this bud dies gradually, the leaves withering in place and persisting strongly (fig. 7, *B*). The local necrotic lesion may be pruned out, and thereafter the remaining parts of the plant appear free from the disease. Mme. Butterfly has been held for 4 years after the appearance of such lesions, showing no further symptoms. The disease can be induced in such plants in typical form upon reinoculation. Such reinoculation has been uniformly successful in eight attempts in Briarcliff, six in Mme. Butterfly, and one in Ophelia. The second inoculations followed the first after 9 to 18 months, when the first symptoms were apparently inactive. In every instance the typical necrotic effects developed after the second inoculation with no recognizable departure from the effects of the first inoculation. Failure to protect against primary lesions upon reinoculation is usually regarded as evidence that the virus is localized rather than systemic. Such evidence supports the writers' conclusions, from subinoculations and from successful pruning out of streak lesions, that the necrotic effects of streak in hybrid teas such as these are primary local lesions.

A third group of varieties, long considered immune, includes C. F. Meyer, Duchess of Wellington, Joanna Hill, Kaiserin Auguste Viktoria, Margaret McGredy, Ragged Robin, *Rosa hugonis*, *R. nutkana*, Souvenir de Claudius Pernet, and Ulrich Brunner. These varieties developed neither primary necrosis nor systemic symptoms when inoculated with buds of systemically affected varieties or when budded into canes of streak-affected Odorata in routine trials. Several attempts to recover streak by subinoculation failed. However, after long union with streak-affected scions of Silver Moon this apparent immunity broke down in Duchess of Wellington, Kaiserin Auguste Viktoria, and *R. nutkana*, and these varieties expressed systemic symptoms. It appears that the incubation period may be greatly prolonged in some varieties, and also that passage of the streak virus upward across

a union may be far slower than the downward movement. It is therefore necessary to examine the evidence for resistance or immunity in these varieties in further detail.

Two large plants of Odorata naturally infected with streak were budded at various times with C. F. Meyer, Duchess of Wellington, Margaret McGredy, Mme. Butterfly, *Rosa hugonis*, Silver Moon, Souvenir de Claudius Pernet, and Talismân. The Odorata plants showed cane symptoms and also brown leaf markings and senescence patterns of systemic infection at various times during this test. Mme. Butterfly shoots died 4 months after the bud was set, with characteristic necrotic streak symptoms. A Talisman bud developed into a scion branch 8 inches long in 2 months, remained apparently healthy for 4 months, but died with necrotic streak symptoms during the sixth month of contact. Two Silver Moon buds, set one in each plant of Odorata, developed into scion branches about 3 feet long before systemic symptoms appeared in the fourth month. Thus the streak virus is shown to have passed upward, inducing typical necrotic and typical systemic effects in appropriate varieties in 4 to 6 months after budding into streak-affected Odorata. *Rosa hugonis* grew on the streak-affected Odorata for 13 months without showing symptoms, and Margaret McGredy grew for 23 months, developing a strong well-branched shoot 3 feet tall; but no symptoms were expressed, and a subinoculation to Mme. Butterfly at the end of the test was negative. Souvenir de Claudius Pernet grew normally on streak Odorata for 21 months, and subinoculations to Briarcliff and Mme. Butterfly after 8 months were negative.

C. F. Meyer was set in streak Odorata on June 7, 1934, and Duchess of Wellington on November 8, 1934. Both developed vigorous scion branches which remained symptomless until the test was concluded September 28, 1937. On November 1, 1935, healthy Mme. Butterfly was budded into these scions, providing a susceptible test variety separated from the systemically affected Odorata by intermediate canes of C. F. Meyer 30 inches long and Duchess of Wellington 17 inches long. Mme. Butterfly grew vigorously, flowered freely, and ripened fruits, without showing streak effects in 23 months, when thus separated from the streak source by intermediate scions. Mme. Butterfly budded at the same date into Silver Moon, an intermediate susceptible scion on the same Odorata stock, was killed during the third month by the usual black necrotic effects of streak in this variety. This experiment seemed to provide strong evidence that Duchess of Wellington and C. F. Meyer are immune to streak. No further tests were made with the latter variety, but in a parallel trial streak-affected Silver Moon set in a cane of Duchess of Wellington induced typical systemic streak in Duchess of Wellington in 14 months. During this time the Silver Moon scion attained a length of several feet and expressed strong streak symptoms. Subinoculation from Duchess of Wellington to Mme. Butterfly was positive. The correct interpretation of this evidence would appear to be that Duchess of Wellington is susceptible but offers some resistance to streak invasion, particularly to the movement of the streak virus upward across a union.

Like Duchess of Wellington, Kaiserin Auguste Viktoria and *Rosa nutkana* developed systemic streak symptoms after long delay when inoculated with buds of streak-affected Silver-Moon. Both these

varieties showed streak 15 months after budding. The inoculated plant of *R. nutkana* carried a healthy scion shoot of Mme. Butterfly on a separate cane from that budded to streak. Necrotic spotting and gradual dieback of Mme. Butterfly became evident 12 months after the Silver Moon bud was inserted; i. e., evidence of systemic spread of the virus was available 3 months before *R. nutkana* expressed symptoms. The well-established Mme. Butterfly scion, 18 months old when the inoculation was made, was killed completely by streak necrosis, and some dieback effects appeared in *R. nutkana* also. Streak infections have been demonstrated by subinoculation before symptoms were evident in other systemically susceptible roses, e. g., Multiflora and *R. wichuraiana*, in routine trials.

Joanna Hill was twice budded with streak from Silver Moon without expressing symptoms. One inoculated plant was held in the greenhouse for 18 months; the other was grown for a year in the field after 4 months' contact with streak in the greenhouse. Ragged Robin was similarly budded twice, one plant was held under greenhouse conditions for 12 months and the other was moved to the field after 3 months' incubation in the greenhouse. No evidence of streak appeared in Ragged Robin. *Rosa hugonis* carried a strong streak-affected Silver Moon scion shoot for 41 months without showing streak, and subinoculation from *R. hugonis* to Mme. Butterfly after 34 months was negative. Ulrich Brunner carried a vigorous streak-affected Silver Moon scion shoot for 24 months without developing streak symptoms and a Mme. Butterfly scion remained healthy on a separate cane. Five plants of Souvenir de Claudius Pernet were inoculated with streak from Silver Moon and were held for 5 months (1 plant), 7 months (2 plants), 11 months (1 plant,) and 22 months (1 plant) without developing streak symptoms.

The data here detailed require the transfer of Duchess of Wellington, Kaiserin Auguste Viktoria, and *Rosa nutkana* to the group of roses subject to systemic invasion by streak. There is further serious doubt as to whether true immunity to streak occurs, since severe and prolonged testing would be required to establish this point. Nevertheless there is good reason to expect that these varieties will prove at least resistant to streak if they chance to be budded or grafted on streak-affected understocks in the usual commercial practice.

Considerable evidence is available that mosaic and streak move slowly through old canes, and this fact would account for the long incubation periods in the varieties that grew sluggishly after budding. A peculiar instance of failure of the streak virus to move upward from roots may be of interest. Healthy scions of Silver Moon were grafted on pieces of roots of streak-affected Odorata, by the method known as piece-root grafting, on March 6, 1935. The object was to demonstrate the occurrence of streak virus in roots of roses. Two scions developed into vigorous plants but showed no symptoms of streak during 13 months in the greenhouse. They were then transplanted to the field and grew an additional 20 months without evidence of streak infection. On December 10, 1937, 33 months after the root grafts were made, the two plants were dug and sample buds and pieces of root handled as buds were set separately in four healthy Mme. Butterfly plants. No streak developed about the buds, but typical streak lesions developed in each Mme. Butterfly plant "budded" with pieces of root. Symptoms were evident in the

ourth month and girdling was complete in the eighth month after ›udding. Thus streak was recovered from the roots of each plant ›ut from the canes of neither. The presence of the streak virus in ose roots was thus demonstrated by a method more devious than vas originally intended. It was not expected that for a period of 33 nonths the virus would fail to move upward from the roots into hoots of the Silver Moon variety, which is known for tremendous 'igor.

The writers have stressed the evaluation of symptoms and the ›revalence of these diseases in roses. It has not been practicable to .ttempt an investigation of transmission of the viruses to plants in ther genera of the Rosaceae. It is not unlikely, especially in the ght of Thomas' (*16*) work, that other members of this family may e susceptible to one or more of these viruses.

VIRUS INTERACTIONS

The evidence at hand is inadequate to determine whether the viruses iscussed in this paper are separate entities or strains of a general rose ıosaic group. Typical rose mosaic and the yellow variants agree ıther closely in general symptom types produced, in incubation eriod, in failure of juice inoculations to transmit, and in known host ınges. The streak disease, on the other hand, differs sharply from ıe mosaic diseases in symptoms and in varietal responses, but is milar with respect to incubation period and modes of transmission › far as is known.

The results of a number of double inoculations may be discussed t this point since the interaction of viruses has been widely used in ıterpreting relationships. On October 1, 1937, buds of Talisman ırrying yellow mosaic of the Talisman strain were set in five Mme. utterfly plants that had carried typical rose mosaic for a year or ıore. During the following 10 months the yellow strain was ob- 'rved to dominate the green type in two plants, but symptoms of the 'een type were not entirely suppressed. In the other three plants ıe green pattern continued unchanged with no effect from introduc- on of the second virus. The Margaret McGredy strain of yellow osaic introduced into two mosaic-affected Mme. Butterfly plants ıder like conditions produced no effect on the symptoms expressed ıring the succeeding 10 months. Yellow mosaic from Margaret cGredy was successfully superimposed on yellow mosaic of the ish Charm strain in the former variety in a single trial. The cond inoculation followed the first by 28 months, and the more 'illiant pattern of the second virus was recognizable 4 months later.

single attempt to superimpose the Talisman strain on the Irish harm strain in the Talisman variety evidently failed. The second rus was inoculated 28 months after the first; no change in response ıs noted 8 months later.

Typical rose mosaic in Mme. Butterfly, yellow mosaic in Talisman, d healthy Silver Moon and Kaiserin Auguste Viktoria were budded to each of two large Odorata plants in pots on November 1, 1935. ıckers of Odorata were permitted to develop to express the symp- m reaction of this stock, and the five-variety system was observed intervals for nearly 30 months. There was no clear evidence of

Silver Moon expressed symptoms of each virus in each of the two plants; Talisman and Kaiserin Auguste Viktoria showed only yellow mosaic symptoms in one plant and no symptoms in the other. Both mosaic and yellow mosaic expressed prominent symptoms at intervals, one seeming to dominate in one variety, the other in another. At the conclusion of the test both viruses were evidently present in Mme. Butterfly, Odorata, and Silver Moon, in some instances symptoms of both appearing in the same leaf. The logical interpretation would be that the two can exist and multiply together without marked effect on each other. Since both are systemic, this evidence has no important bearing on their relationship, but if it has weight at all, it is against their being strains of a single virus.

Yellow patterns suggesting a mutation from the green to a yellow type were observed in two Mme. Butterfly plants affected with typical rose mosaic, one in the field and one in the greenhouse. Subinoculation from each to healthy Mme. Butterfly reproduced only green mosaic. The possible origin of yellow types from the typical rose mosaic by mutation was therefore not confirmed.

More significance is attached to the production of the primary cane lesions of rose streak in varieties systemically affected with typical rose mosaic. The varieties Briarcliff and Templar each show systemic symptoms of rose mosaic, and each is subject to primary necrosis about the streak-affected bud when the latter disease is introduced. Streak was produced in Briarcliff twice when introduced 4 months after, and 5 months after, mosaic inoculation. Streak symptoms appeared in 27 days in typical form in each plant and remained localized. Mosaic symptoms were unaffected by introduction of the second virus during 14 months' subsequent observation. Similarly, streak was superimposed on mosaic in a Templar plant of long-standing mosaic history. Typical streak developed in 10 weeks, ran its course in 6 months, and was pruned out, leaving typical mosaic effects still evident. The fact that Briarcliff and Templar, systemically infected with the rose mosaic virus, expressed the characteristic primary lesions of streak on subsequent budding is an indication that the streak virus is not closely allied to that of rose mosaic.

SPREAD OF ROSE MOSAIC IN NURSERY PRACTICE

Observers agreed soon after rose mosaic was first recognized that no natural spread occurred under glass. The suspicion that natural spread might occur in the field has been more persistent, but so far as the writers are aware, nearly all field evidence of spread is based on the appearance of crinkle and speckle types herein shown to be unrelated to rose mosaic. The appearance of mosaic in new varieties of roses of comparatively recent seed origin and the occasional presence of high percentages of the disease in certain nurseries would suggest natural spread of authentic rose mosaic. The writers' test plantings in Oregon, New York, Virginia, and Maryland have failed to yield any evidence of natural spread, and all attempts to transmit the disease by means of insects have failed. In the writers' opinion all available evidence for spread of rose mosaic can be accounted for by propagation practices.

No evidence was furnished by the trials described above that rose mosaic spreads in the field in commercial rose nurseries in Oregon or

in a general farming area at Babylon, N. Y. Nor was there eviden of spread in 1933 at Arlington, Va., when 15 healthy Mme. Butterf plants were grown near mosaic sources throughout the season and th returned to the greenhouse for observation. Again in 1934, 52 healtl Briarcliff, 31 Mme. Butterfly, 3 Ophelia, 15 Rapture, and 2 Canin plants showed no spread of mosaic from adjacent proved source plan at the Arlington farm. At Beltsville, Md., 34 plants of Radianc 5 plants each of Briarcliff, Canina, Joanna Hill, Mme. Butterfl Manetti, Multiflora, Odorata, and Texas Wax, 1 plant of Ragg Robin, and 2 of *Rosa hugonis*, grown adjacent to proved mosai yellow mosaic, and streak source plants from March or April 19 through September 1936, showed no spread of any of these thr diseases. In view of the fact that several of the varieties included these trials are known to express mosaic symptoms with a hig degree of reliability, these results offer strong evidence that spread mosaic does not commonly occur in these localities. A separate te for natural spread of rose streak at Beltsville was also negative. N spread of the disease from affected plants to 80 plants of healtl Silver Moon, a readily affected variety, could be detected at any tin during a 4-year exposure.

It is well known that mosaic is carried in cuttings or scions tak from affected plants. However, field-budding practices as a means pseudo-natural spread seem to have been overlooked. Two gener practices for maintaining a supply of Manetti wood for cuttings a followed in the West. In the first, a "mother block" is maintain as a source of cuttings, and hundreds of cuttings are rooted from ea established plant each year that it is in use. Thus scions are nev worked on the plants in the mother block that provides the cutting

In the second procedure, the mother block is eliminated and cuttin are collected at random from the tops of field-budded plants wh the latter are cut back to force the inserted buds. Such cuttings fro budded plants are subject to the hazard of contact with buds th may have carried mosaic. In each practice the Manetti cuttings a lined out in the field for rooting, and are budded the following summ Should a mother-block plant become infected, all its vegetative pro eny would carry the mosaic virus and transmit it to previously healtl buds worked on them. However, if the mother block is free fro mosaic, the disease will appear only in plants grown from infect budwood. When cuttings are taken from budded plants, any cuttin from a diseased plant may transmit the disease to healthy insert buds in the next season. Mosaic bud stock thus infects the healtl bud stock of the following season. Such infection is avoided by th mother-block system. The practice of taking cuttings for understocl from the tops of budded plants is doubtless a sound commercial pra tice from other viewpoints, but it provides an efficient means of pre ervation and dissemination of virus diseases such as rose mosaic, yello mosaics, and, in varieties subject to systemic invasion, rose strea As far as the writers have been able to determine, the higher percen ages of mosaic recorded in table 4 are from nurseries that root cutting from budded plants. In view of the difficulty of recognizing mosa in Manetti and Ragged Robin stocks as well as in a large number budded varieties, it is manifestly impossible to recognize the presen of rose mosaic in affected understocks propagated from budded plant A high percentage of infection can thus be built up without conspic

ous symptom expression. When a variety capable of expressing strong mosaic symptoms is worked into such understocks, a sudden appearance of mosaic may result, and will usually be attributed to natural spread. The practice of taking cuttings from budded stocks is so well suited to preservation of the virus diseases and to their dissemination to new varieties that it seems adequate to account for all observed spread of rose mosaic.

CONTROL

The control measures outlined below are those indicated by the writers' evidence on the nature and behavior of the diseases here discussed. Tests of these measures on a nursery scale have not been made.

Practical control of mosaic in propagation on Manetti understocks can be accomplished by establishing a disease-free mother block. This is desirable since mosaic in Manetti stocks (and others) is difficult to diagnose in the field. A small block of plants can be checked by budding to varieties readily diagnosed when affected, such as Mme. Butterfly, Ophelia, or Rapture, and plants thus proved healthy can thereafter be reserved as healthy mother plants. The test variety may be budded into the canes of the mother block in the field, or buds from the mother block may be set in canes of the test varieties. If both stock and scion grow vigorously, a period of 3 or 4 months is adequate for a test. A mosaic-free mother block may be expanded and renewed as desired by means of cuttings. With such a proved source of healthy cuttings available, mosaic will occur only as a result of using infected buds, and will not spread to other varieties.

Selection of budwood from healthy plants is also important. This is easily feasible in the varieties that are severely injured when affected. In those varieties in which mosaic symptoms are more difficult to detect, affected buds sometimes may be selected even by competent propagators. Little harm will result from this, however, provided the mother-block system is used. The use of this system should be feasible for other stocks as well as Manetti.

The procedure outlined for control of mosaic is suggested for the control of the yellow mosaics and of streak. The varieties Mme. Butterfly, Ophelia, or Rapture express yellow mosaics clearly; buds of these varieties die when set in streak-affected stocks.

The possibility remains that some of these diseases may be spread by insect vectors in some localities or in some seasons, but there is no evidence of such spread at present. Control recommendations are therefore confined to elimination of known modes of propagation of these diseases.

DISCUSSION

Slow movement of the mosaic and streak viruses in inoculated plants and slow expression of symptoms are not believed to weaken the validity of the writers' index trials, since responsive test varieties were used and since tests were not considered conclusive unless scion and stock grew actively. If others using the index method will observe the same precautions, conclusive readings may be expected within 3 or 4 months. Prolonged delay in the expression of symptoms of both mosaic and streak was observed in varieties that express symptoms sporadically after infection is fully systemic. In such varieties the

physiology of symptom expression is involved as well as the physiology of virus movement. Where movement of the virus has been demonstrated to be slow by subinoculation trials, it seems explainable according to established principles laid down by Bennett (*2*) and others.

It has been suggested above that White (*23*) apparently had a yellow mosaic, and possibly streak, in his collections. The rose virus disease described by Valleau (*18*) and by Johnson and Valleau (*9*) is apparently typical rose mosaic in a variety that produces only the watermark-ring series of symptoms. The last-named authors have suggested that their collection is a specimen of White's rose mosaic. Milbrath's (*12*) albication seems to agree in symptoms with one of the writers' yellow mosaic collections, although nontransmissible albication patterns occur in roses. Thomas (*16*) illustrates a pattern closely similar to typical rose mosaic induced in the Belle of Portugal variety by transfer from mosaic-affected apple. Christoff (*6*) mentions a mosaic of *Rosa gallica* L. transmissible to apple and pear. Unfortunately the writers do not have symptoms of their viruses in these roses for comparison.

The streak disease, which produces necrotic effects on some rose varieties, appears to differ from wilt and dieback, described by Grieve (*8*) from Australia, and from Gigante's (*7*) new virus of rose in Italy, both in symptoms produced and in modes of transmission. Both Grieve and Gigante stress symptoms in young leaves, whereas streak symptoms are recognizable only as the leaves approach full size and after. Moreover, both the Australian and the Italian viruses are juice-transmissible, and the former is filterable. Gigante considers his disease distinct from Grieve's and from rose mosaic.

SUMMARY

The symptoms of rose mosaic in Mme. Butterfly are prominent hlorotic areas feathering away from the midribs of leaflets, and also ing, oak-leaf, and watermark patterns. Various combinations of hese symptoms, with various degrees of intensity, are expressed in ther rose varieties and species. Some rose varieties and the undertocks Manetti, Ragged Robin, and Texas Wax express symptoms oo irregularly for accurate direct diagnosis.

Yellow mosaics are characterized by brighter and lighter yellow atterns than are found in typical rose mosaic.

The streak disease of roses produces brown rings, brown or yellowsh vein banding, and senescence patterns in leaves, and brownish r greenish ring markings in canes. Certain hybrid tea roses are ubject to necrotic primary lesions in canes.

Many chlorotic speckle and crinkle patterns, including the disorder ı Manetti known as rattlesnake in the Northwest, are unrelated to ose mosaic, and have not been shown to be of virus etiology.

Field diagnosis of mosaic and streak is unreliable because of the endency of the symptoms to become masked in some varieties.

The occurrence of rose mosaic has been confirmed by the transfer om 17 varieties of roses from 7 States and the Province of Ontario, ut the disease has been found to be prevalent only in association ith certain propagation practices. Index tests of understocks from regon, California, Texas, and elsewhere show that the disease is ss prevalent than was formerly claimed.

Yellow mosaics have been found in scattered single plants in four localities.

Streak is known from Maryland, New York, Texas, Virginia, and the District of Columbia. This disease affected 30 percent of the roses in one collection of varieties.

Mosaic, yellow mosaics, and streak have been transmitted only by tissue union, i. e., by various styles of budding or grafting. No transmission resulted when buds were removed before union occurred. The minimum incubation periods recorded for the three virus types are all of the order of 20 days. In less responsive varieties and under less favorable conditions incubation may require several months.

No seed transmission of mosaic or streak was evident in small populations of seedlings from affected plants.

No evidence of insect transmission was obtained from trials of 31 species of insects for mosaic nor from tests of 34 insect species for streak.

Strawberry crinkle was not transmitted to rose by *Philaenus leucophthalmus* (L.) nor by *Capitophorus fragaefolii* (Ckll.). Red raspberry mosaic was not transmitted from red raspberry to rose by *Amphorophora rubi* (Kalt.), nor aster yellows from China aster to rose by *Macrosteles divisus* (Uhler).

All roses adequately tested have proved susceptible to rose mosaic and yellow mosaics, but differences are found in intensity of symptom expression, i. e., in degree of tolerance. Rose varieties and species fall into three groups with respect to streak reactions: (1) Those susceptible to systemic infection, (2) those subject to primary necrotic lesions in canes, and (3) those apparently resistant or immune to infection.

No natural spread of mosaic or streak has been detected in field trials in Maryland, New York, Oregon, and Virginia. It is indicated that the nursery practice of propagating roses on rooted cuttings taken from the tops of budded understocks of the previous season is the probable mechanism of the apparent spread of rose mosaic in nature.

It is suggested that rose mosaic, and probably also yellow mosaics and streak, may be controlled by using understocks derived from a mother block indexed to assure freedom from these diseases.

The available evidence is considered inadequate for determining whether rose mosaic and five yellow mosaics are closely allied. The streak virus is believed to be unrelated to the mosaics and to be unlike the necrotic diseases of rose described by Grieve from Australia and by Gigante from Italy.

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EFFECT OF DAY LENGTH AND TEMPERATURE ON THE FLOWERING AND GROWTH OF FOUR SPECIES OF GRASSES[1]

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INTRODUCTION

In attempts to carry on physiological investigations with native grasses in the greenhouse during the winter months, the writer has often encountered difficulty in obtaining flower production and good vegetative growth. Some of the grasses when grown under winter greenhouse conditions produce a low tufted top growth and never send up flower stalks. Other species produce only a few leaves and then seem to stop growing until late in the winter or early spring. As a result of the work of Garner and Allard (*4, 5, 6*),[2] Tincker (*9, 10*), Adams (*1, 2, 3*), Gilbert (*7*), Thompson (*8*), and others, it was thought that lengthening the day or varying the temperature might bring about increased growth and production of flower stalks in those species that usually do not grow well in the greenhouse during the winter.

In this paper are presented the results of experiments on the effects of different day lengths and temperatures on bluestem (*Agropyron smithii* Rydb.), locally known as western wheatgrass, blue grama (*Bouteloua gracilis* (H. B. K.) Lag.), bluejoint turkeyfoot (*Andropogon furcatus* Muhl.), locally known as big bluestem, and switchgrass (*Panicum virgatum* L.). The first two species are the most common grasses in the short-grass plains and make fairly good growth but rarely flower in the greenhouse during the winter season. The last two species are common in the tall-grass prairies and, at least at Cheyenne, Wyo., make very little growth under winter greenhouse conditions.

METHODS

Three series of experiments were carried out, the first and third during the winter when the days are naturally short and greenhouse temperatures can be kept low, and the second in the summer when the days are naturally long and greenhouse temperatures become high during the day. In the first series plants of *Agropyron smithii* and *Bouteloua gracilis* were grown in seed flats 16 by 24 by 4 inches, 100 plants to a flat, from October 21, 1934, to March 21, 1935. The plants in this series (series 1) were grown in 8-hour, 20-hour, and natural day lengths at a temperature of 60° F. and in a natural day length at a temperature of 75°. In the second series, plants of the same two species

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[2] Italic numbers in parentheses refer to Literature Cited, p. 670.

were grown in seed flats, 77 plants to a flat, in an 8-hour and a 20-hour day, from June 11, 1935, until October 4, 1935. During this period the temperature was maintained as close to 80° as possible, although during the middle of the day it was often several degrees higher. The third series contained plants of *Agropyron smithii*, *Andropogon furcatus*, *Bouteloua gracilis*, and *Panicum virgatum*. These plants were grown in seed flats from November 20, 1936, to March 24, 1937. The plants were all thinned to 50 per flat and grown under the following six conditions, with duplicate flats of each species placed in each treatment.

(1) In a natural, or short, day length at a day and night temperature of 75° F.

(2) In a natural day length at a day temperature of 75° F. and a night temperature of 60°.

(3) In an 18-hour, or long, day length at a day and night temperature of 75° F.

(4) In an 18-hour day at a temperature of 75° F. during the day and a night temperature of 60°.

(5) In a natural day length until January 20 and then in an 18-hour day length until March 24, at a day and night temperature of 75° F.

(6) In an 18-hour day length until January 20 and then in a natural day length until March 24 at a day and night temperature of 75° F.

The desired temperatures in the three series of experiments were maintained by hand operation of the greenhouse ventilators. This method admittedly allows for some variation, but except for the summer series this was never more than 5° F., according to the thermograph records that were kept. The night temperature of 60° used in series 3 was obtained by removing the plants from the greenhouse to a propagating house for the required length of time.

The 18- and 20-hour day lengths were obtained by burning incandescent lamps from shortly before sundown for as many hours as were necessary to give the required day length. The intensity of the radiation of the incandescent lamps at the surface of the soil was 35 foot-candles. The areas lighted by the lamps were screened so as to prevent any stray radiations from reaching areas that were to be kept dark. The 8-hour day length was maintained by covering the plants with a lightproof canvas from 4 p. m. until 8 a. m. daily. This canvas was suspended on a frame 54 inches high, closed at either end by wall-boarding that could be removed during the day. It was not possible to observe any light in the compartment thus formed. The temperature inside never exceeded that of the rest of the greenhouse by more than 2° F.

By thoroughly mixing the soil before placing it in the flats in all three experiments, care was taken to insure that no differences in the soil existed between treatments.

According to information supplied by the United States Weather Bureau, the average length of daily sunlight during the time the plants were being grown in the winter was about 10¼ hours. Thus the natural day length in the winter will be considered a short day.

The dry weight per plant was used as the criterion of growth of the plants subjected to the various treatments. The dry weights were determined by carefully washing the soil from the plants, separating the roots from the tops, placing both in an oven at 100° C. for an hour, and then drying them to constant weight at 80°.

EXPERIMENTAL RESULTS AND DISCUSSION

EFFECT OF DAY LENGTH AND TEMPERATURE ON FLOWERING

The number of days elapsing between the date of planting and the date on which at least three plants in a given treatment showed flower stalks are given in table 1. The results presented here indicate that *Agropyron smithii* is a long-day plant and that the proper temperature alone will not bring about flowering. This is brought out by the fact that under the conditions of these experiments plants of this species flowered in an 18- or 20-hour day when the temperatures in which they were growing ranged from 60° to 80° F. but failed to bloom when growing at these temperatures in an 8- or 10-hour day. It will be noted that plants of this species in series 3 failed to bloom in an 18-hour day when the night temperature was lowered from 75° to 60°. This failure to flower may have been due entirely to the low temperature, but since the plants of this series that did flower did so on the last day of the experiment it seems possible that the plants were not grown long enough to reach the flowering stage.

TABLE 1.—*Number of days between the date of planting and the appearance of first bloom of different grasses grown in different day lengths and temperatures*

Treatment		Time from planting to blooming in indicated species			
Day length	Temperature conditions	*Agropyron smithii*	*Andropogon furcatus*	*Bouteloua gracilis*	*Panicum virgatum*
	°*F.*	*Days*	*Days*	*Days*	*Days*
Series 1 (winter):					
8-hour day	60	[1]		[1]	
Natural, or 10-hour, day	60	[1]		[1]	
20-hour day	60	149		[1]	
Natural, or 10-hour, day	75	[1]		93	
Series 2 (summer):					
8-hour day	80	[1]		72	
20-hour day	80	98		100	
Series 3 (winter):					
Natural, or 10-hour, day	75 throughout	[1]	[1]	70	72
Natural, or 10-hour, day	*75 day; 60 night*	[1]	101	[1]	93
18-hour day	75 throughout	120	[1]	113	[1]
18-hour day	75 day; 60 night	[1]	[1]	[1]	[1]
10-hour, 2 months; 18-hour, 2 months.	75 throughout	[1]	[1]	70	[1]
18-hour, 2 months; 10-hour, 2 months.	75 throughout	[1]	[1]	87	[1]

[1] Failed to bloom.

As shown in table 1, plants of *Bouteloua gracilis* flowered in an 18- and 20-hour day as well as in an 8- or 10-hour day when the temperature remained at 75° F. or above, but failed to flower in any day length when the temperature dropped to 60° for even a few hours daily. The plants grown in the shorter day lengths flowered earlier than those grown in the longer day lengths. These results indicate that *B. gracilis* is an indeterminate type of plant as far as its blooming response to day length is concerned and that a fairly high temperature is necessary to produce flowering.

Plants of *Andropogon furcatus* bloomed in the 10-hour day at a temperature of 75° F. during the day and 60° during the night, but

did not bloom in this short day when the temperature was kept at 75° throughout, nor did the plants of this species bloom in the 18-hour, or long, day. These results seem to indicate that *A. furcatus* is a short-day plant and that temperature alone does not determine whether it will flower. The plants of this species grown at a temperature of 75° in the short day may not have flowered because of the extremely small growth they made, having a total dry weight of only 23 mg.

Plants of *Panicum virgatum* bloomed when grown in a short day, regardless of the temperature, although lowering the temperature at night retarded the blooming date somewhat. This would indicate that *P. virgatum* is a short-day plant and that the temperatures used in this experiment have little influence in determining whether or not these plants will flower.

In setting up the treatments in which the plants were grown, first in a short day and then in a long day or first in a long day and then in a short day, it was thought that the day length unfavorable to flowering when applied first would cause the plants to vegetate and that then changing to the day length favorable for flowering would result in these plants coming into flower very rapidly. However, it will be noted from table 1 that only the plants of *Bouteloua gracilis* flowered in these treatments and that the plants of this species were able to lower in either a continuous long or a continuous short day. These esults seem to show for the other three grasses under the conditions of these experiments that the effect of growing them for 2 months n a day length unfavorable for blooming was not overcome by growng them afterward for 2 months in a day length favorable for blooming. They also show that a day length unfavorable for blooming was ible quickly to overcome the effects of growing the plants for 2 months n a day length favorable for blooming. For example, plants of *Panicum virgatum* growing in a long day did not flower while those growing in a short day flowered in 72 days. Yet when plants growing n the short day for 61 days were placed in a long day they did not lower, nor did the plants of this species flower when transferred to a hort day after being in a long day for 2 months.

The classification of *Agropyron smithii* as a long-day plant, *Andropogon furcatus* and *Panicum virgatum* as short-day plants, and *Bouteloua racilis* as a plant of indeterminate day length is borne out by the ime of the year in which these plants come into flower when growing inder natural conditions. Plants of *A. smithii* bloom in the latter art of June when the days are longest. Plants of *B. gracilis* will end up flower stalks throughout the summer. Plants of *A. furcatus* nd *P. virgatum* come into flower around the middle of August, when he days are becoming shorter.

EFFECT OF DAY LENGTH AND TEMPERATURE ON GROWTH

The dry-weight determinations on the plants of the first series ere ruined by an overheated oven and so cannot be presented. The ry weights per plant of the roots, tops, and total plant of the species rown in series 2 and 3 are shown in tables 2 and 3, respectively. he values presented in table 3 are the means of the two replications f each species in each treatment. In both series, with one exception, e weights of the individual parts of a plant of one species always

varied in the same direction with change in treatment. Thus, if the roots of the plants of one species increased when the day length was increased, the tops also increased in dry weight and naturally the entire plant showed the same trend. This makes it possible to discuss together instead of separately the results of the treatments of the plants of the different species.

TABLE 2.—*Dry weight per plant of Agropyron smithii and Bouteloua gracilis (series 2) grown in the summer of 1935 under different day lengths at 80° F.*

Species and treatment	Dry weight per plant: Root	Top	Total
	Gram	*Gram*	*Gram*
Agropyron smithii:			
8-hour day	0.056	0.131	0.187
20-hour day	.053	.124	.177
Bouteloua gracilis:			
8-hour day	.255	.436	.691
20-hour day	.289	.620	.909

TABLE 3.—*Dry weight per plant of grasses of series 3 when grown in different day lengths and temperatures*

Species and plant part	Dry weight per plant under indicated conditions: Normal or 10-hour day: 75° F. throughout	Normal or 10-hour day: 75° F. day, 60° F. night	18-hour day: 75° F. throughout	18-hour day: 75° F. day, 60° F. night	10-hour day, 2 months; 18-hour day, 2 months; 75° F. throughout	18-hour day, 2 months; 10-hour day, 2 months; 75° F. throughout
Agropyron smithii:	*Gram*	*Gram*	*Grams*	*Gram*	*Gram*	*Gram*
Roots	0.176	0.235	0.194	0.228	0.236	0.243
Tops	.312	.420	.346	.372	.408	.375
Total	.488	.655	.540	.600	.644	.618
Andropogon furcatus:						
Roots	.009	.023	.311	.324	.030	.113
Tops	.014	.033	.545	587	.098	.155
Total	.023	.056	.856	.911	.128	.268
Bouteloua gracilis:						
Roots	.184	.091	.315	.206	.235	.323
Tops	.560	.218	.825	.571	.731	.652
Total	.744	.309	1.140	.777	.966	.975
Panicum virgatum:						
Roots	.010	.074	.299	.223	.024	.101
Tops	.006	.053	.721	.504	.052	.063
Total	.016	.127	1.020	.727	.076	.164

The results of the dry-weight determinations (table 2) indicate that day length has little effect on the growth of plants of *Agropyron smithii*, provided the temperature is kept constant. This is brought out by the fact that in series 2 the plants of this species had about the same dry weight when grown in either the long or the short day and that in series 3, when grown at a temperature of 75° F. throughout, the plants in the long day had a slightly greater dry weight than those

in the short day but, when grown at a night temperature of 60°, the plants in the short day had a greater dry weight. The variations obtained between replications in series 3 indicate that not much importance can be attached to the differences noted between the dry weights of the plants growing in the different day lengths at the same temperature.

The results presented in table 3 also indicate that reducing the temperature at night causes an increase in the growth of plants of *Agropyron smithii*, since the plants growing in the low night temperature had the greater dry weight in both day lengths. This increase may be due to the reduced respiration rate occurring at the lower temperature, which would mean that less of the material manufactured during the day would be respired away at night. It can be seen that the plants in the short day showed a greater increase in dry weight when subjected to the lower temperatures than did the plants in the long day. This may be due to the fact that the former were in the low temperature for about 14 hours daily and the latter for only 6 hours daily. No explanation can be offered as to why the plants of *A. smithii* that were grown in a short day and then in a long day or were grown in a long day and then in a short day had somewhat greater dry weights than the plants grown continuously in a long or a short day.

The results of the dry-weight determinations on plants of *Andropogon furcatus* (table 3) require little discussion. They show that increasing the length of day greatly increases the growth or dry weight of the plants of this species, regardless of the temperature used. They also indicate that lowering the night temperature increases the dry weight of the plants, although not to the extent that increasing the day length does. This increase in dry weight in the lower night temperature might possibly be explained on the basis of reduced respiration rate, as in the case of plants of *Agropyron smithii*.

The results of the dry-weight determinations on plants of *Bouteloua gracilis* (tables 2 and 3) show that plants of this species are capable of making fairly good growth in either a long or a short day, but that *increasing the day length markedly increases the growth of the plants,* provided the temperature remains constant. The fact that the dry weights of the plants grown first in a short day and then in a long day or first in a long day and then in a short day were about intermediate to the dry weights of the plants grown throughout in a long or a short day indicates that a short day length is not so detrimental to the growth of this species as it is to that of *Andropogon furcatus* and *Panicum virgatum*. In the case of these latter species the dry weights of the plants grown in a short day and then in a long day or in a long day and then in a short day were nearer to those of the plants grown entirely in the short day.

It is interesting to note that lowering the night temperature in which the plants of *Bouteloua gracilis* were growing caused a reduction in the dry weight of the plants in both day lengths. It can also be seen that reducing the night temperature reduced by more than half the dry weights of the plants growing in the short day, but reduced by only a third those of the plants growing in the long days. These results would seem to indicate that plants of this species require a higher temperature than those of the other species for their growth

and that lowering the night temperature to 60° F. interferes with their metabolic processes to such an extent that the ensuing reduction in food production cannot be balanced by any reduced respiration rate that may result from the lower temperature. This is further borne out by the fact that the plants growing in the short day, which were subjected to the low temperatures for 14 hours daily, suffered a greater reduction in their dry weight than the plants growing in a long day, which were subjected to the low temperatures for only 6 hours daily. It will be remembered that the plants growing in the low night temperatures were the only ones that did not flower. This would further emphasize the high-temperature requirement of plants of *B. gracilis*.

The results of the dry-weight determinations on the plants of *Panicum virgatum* (table 3) show that the growth of this species is much greater in a long day than in a short day, since the dry weights of the plants under both temperature conditions were much greater in the long day. The results also indicate that lowering the temperature from 75° to 60° F. during the night increases the growth of the plants in a short day but decreases their growth in a long day.

The ratios obtained by dividing the dry weights of the roots by the dry weights of the tops of the plants grown in series 2 and 3 are shown in table 4. These results indicate that day length affects the relative growth of the roots as compared to that of the tops of plants of *Andropogon furcatus* and *Panicum virgatum* but not of *Agropyron smithii* and *Bouteloua gracilis*. Thus the root-top ratios of the former two species were greater in the short days than in the long days under both temperature conditions, but the root-top ratios of the latter two species did not differ significantly in the two day lengths. There is some evidence that changing the plants from a long day to a short day tended to increase the proportion of roots produced more than did changing the plants from a short day to a long day. This is brought out by the fact that the root-top ratios of the plants of all the species worked with were greater when the plants were grown in a long day for 2 months and then in a short day for 2 months than when they were grown in a short day for 2 months and then in a long day for 2 months. However, plants of *A. furcatus* and *P. virgatum* showed much greater differences in root-top ratios between these two treatments than did the plants of *A. smithii* and *B. gracilis*.

TABLE 4.—*Root-top ratio of plants of grasses of series 2 and 3 when grown in different day lengths and temperatures*

Treatment		Root-top ratio of indicated species			
Day length	Temperature conditions	*Agropyron smithii*	*Andropogon furcatus*	*Bouteloua gracilis*	*Panicum virgatum*
Series 2 (summer):	°F.				
8-hour day	80	0.43		0.58	
20-hour day	80	.43		.47	
Series 3 (winter):					
Natural, or 10-hour, day	75 throughout	.56	0.64	.33	1.67
Natural, or 10-hour, day	75 day, 60 night	.56	.70	.42	1.40
18-hour day	75 throughout	.56	.57	.38	.41
18-hour day	75 day, 60 night	.61	.55	.30	.44
10-hour, 2 months; 18-hour, 2 months.	75 throughout	.58	.31	.32	.46
18-hour, 2 months; 10-hour, 2 months.	75 throughout	.65	.73	.50	1.60

The results also indicate that a low night temperature has little o no effect on the relative amounts of roots and shoots produced b the plants, since the root-top ratios of the plants of the four specie did not differ appreciably when grown in a high or a low night ten perature in the same day length.

The plants of *Agropyron smithii, Andropogon furcatus, Boutelou gracilis*, and *Panicum virgatum* after being grown for 11 weeks a

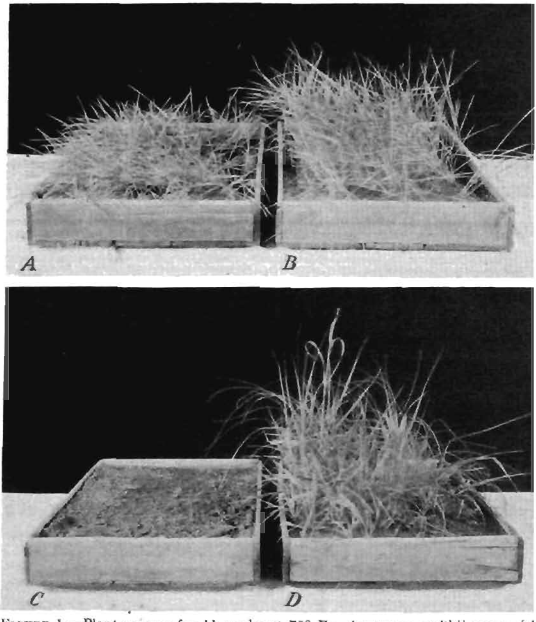

FIGURE 1.—Plants grown for 11 weeks at 75° F.: *Agropyron smithii* grown (A in a 10-hour day and (B) in an 18-hour day; *Andropogon furcatus* grown (C) i a 10-hour day and (D) in an 18-hour day.

75° F. throughout, some in a 10-hour and some in an 18-hour day are shown in figures 1 and 2. The exceedingly poor growth made b the plants *A. furcatus* and *P. virgatum* in the 10-hour day is brough out in figure 1, *C*, and figure 2, *C*. Figures 1 and 2 also show that a long day increased the height of the plants of all four species unde investigation. However, there was no significant difference betwee the dry weights of the plants of *A. smithii* grown in the two treatments

CONCLUSIONS

In general, the results presented in this paper indicate that the naturally short days are, at least in part, responsible for the failure of

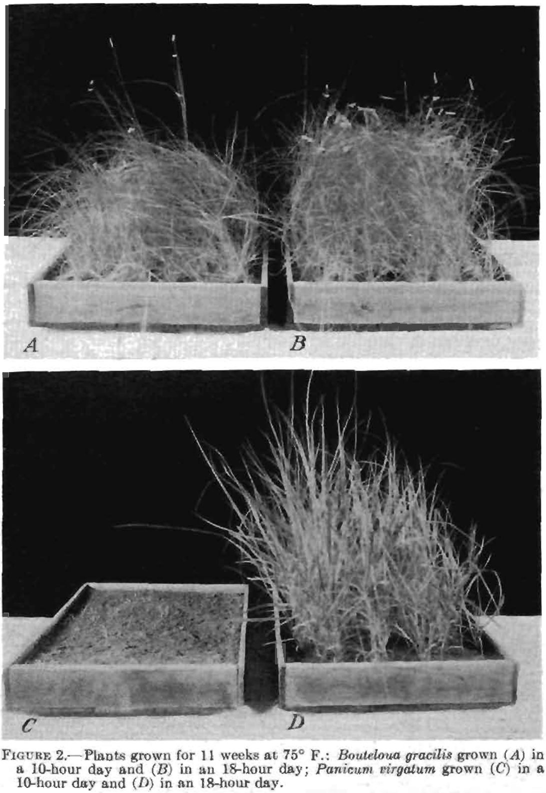

FIGURE 2.—Plants grown for 11 weeks at 75° F.: *Bouteloua gracilis* grown (*A*) in a 10-hour day and (*B*) in an 18-hour day; *Panicum virgatum* grown (*C*) in a 10-hour day and (*D*) in an 18-hour day.

plants of *Agropyron smithii* to bloom and of plants of *Andropogon furcatus* and *Panicum virgatum* to grow well in the greenhouse during the winter. This conclusion is based on the fact that plants of *A.*

smithii bloomed and the plants of *A. furcatus* and *P. virgatum* made good growth when the day length was increased by burning incandescent lamps for several hours each night. The results also tend to show that failure of plants of *Bouteloua gracilis* to flower may have been due to too low temperatures, since the plants of this species bloomed in both long and short days when the temperature was maintained at 75° F. but failed to bloom when the temperature was reduced to 60° for a few hours during the night.

SUMMARY

Plants of *Agropyron smithii* Rydb., *Andropogon furcatus* Muhl., *Bouteloua gracilis* (H. B. K.) Lag., and *Panicum virgatum* L. were grown in the greenhouse under different conditions of day length and temperature. Records were taken on the number of days between planting and the appearance of the first bloom, on the dry weights, and on the root-top ratios of the plants growing under the different conditions.

The results on the number of days required by the plants to flower indicate that *Agropyron smithii* is a long-day plant, *Bouteloua gracilis* is a plant of indeterminate day length, and *Andropogon furcatus* and *Panicum virgatum* are short-day plants.

The dry weights of the plants of *Agropyron smithii* were not affected by the day length, but in the other species the plants growing in the long days had the greater dry weight.

A low night temperature increased the dry weights of the plants of *Agropyron smithii* and *Andropogon furcatus* in both long and short days and also increased the dry weights of the plants of *Panicum virgatum* growing in a short day, but it decreased the dry weights of the plants of *Bouteloua gracilis* growing in both day lengths and also decreased that of plants of *P. virgatum* growing in a long day.

The root-top ratios of the plants of *Andropogon furcatus* and *Panicum virgatum* were greater in the short days than in the long days. No marked difference between the ratios of the plants of the other two species growing in the different day lengths was observed.

There was no significant difference between the root-top ratios of the plants of the four species when grown in a low night temperature and in a high night temperature in either day length.

The results, in general, indicate that day length and temperature markedly affect the growth and flower production of these four grasses and that good growth and blooming can be obtained in the greenhouse in the winter by properly controlling, among other things, these two factors.

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DIGESTIBLE NUTRIENTS OF FEEDING STUFFS FOR THE DOMESTIC RABBIT[1]

By LeRoy Voris, *assistant professor*, Lawson F. Marcy, *instructor*, Edward J. Thacker, *assistant*, and Walter W. Wainio, *instructor*, *Institute of Animal Nutrition, Pennsylvania Agricultural Experiment Station*[2]

INTRODUCTION

The rational feeding of the domestic rabbit has come to be a matter of consequence since the production of this animal as a source of meat for human consumption has attained a permanent and justifiable place in the general food economy.

This report presents the results of the determination of the composition, digestibility, and digestible nutrients of 47 feeding stuffs available for rabbit feeding in the United States. The results of similar investigations by Weiske (*6, 7, 8*),[3] Von Knieriem (*3, 4*), and Brüggemann (*1*), covering an aggegate of 16 roughages and 12 concentrates, are also included for comparative purposes and for the presentation of digestible-nutrient values not given in, but computed from, the papers by Weiske and Von Knieriem.

EXPERIMENTAL SUBJECTS AND EQUIPMENT

The experimental rabbits were young female New Zealand Whites, weighing about 4 pounds each.

The metabolism cages were a standard product having floor dimensions of 18 by 20 inches and a height of 12 inches. The floor of each cage was of ¾-inch mesh No. 12 gage wire, through which the feces dropped onto a ⅛-inch mesh stainless-steel screen, and the urine was directed by a galvanized-iron funnel pan into a bottle below the cage. The stainless-steel screen and funnel pan were free from the body of the cage and were readily removable for the collection of the excreta.

Water was provided outside the cage proper in a cup which fitted over an opening in the door of the cage.

The usual earthenware feeding dishes were found unsatisfactory for quantitative work, since the feed could be scratched out easily. To overcome this difficulty a galvanized-iron feeder with inbent edges was devised and suspended from the door of the cage. This device almost completely prevented the scattering of feed.

A piece of block salt was kept in the cages of the rabbits at all times.

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[2] Substantial assistance in the initiation of this project was received from Dr. Logan J. Bennett, of the U. S. Department of the Interior, Fish and Wildlife Service, in charge of the Cooperative Wildlife Research Unit of the Pennsylvania State College; and a large part of the feeding stuffs used were collected by George S. Templeton, in charge of the U. S. Rabbit Experiment Station, at Fontana, Calif. The writers are also pleased to acknowledge the cooperation of F. W. Burns, extension animal husbandman, Alabama Polytechnic Institute; E. B. Ferris, superintendent of the Holly Springs (Miss.) Experimental Station; Luther Fuller, general farm products agent, T. C. I. & R. Co.; and L. B. Lovett Co., who provided other feeding stuffs used.

[3] *Italic numbers in parentheses refer to Literature Cited, p. 683.*

EXPERIMENTAL METHODS

FEEDING TECHNIQUE

Roughages were cut with a clover cutter and were shaken through a ⅜-inch-mesh screen. The longer pieces thus separated out were returned to the cutter until practically all the material had passed through the screen. The final product was coarse enough to be acceptable to the rabbits, but was relatively free of pieces over 2 inches in length, which they would have wasted.

All of the dry roughages, except bluegrass, were fed alone and the digestibility determined directly. Since the rabbits lost much weight when fed on dried bluegrass alone, this was fed with a commercial rabbit ration and the digestibility determined by difference.

The quantities in which the dry roughages were fed were determined largely by the palatability of the product. The maximum amount of the most satisfactory roughages, such as alfalfa hay, that a rabbit could be trusted to clean up was about 80 gm. per day. In general, the quantity of dry roughage that a rabbit would eat was well below that which would have been required to maintain its body weight.

The green roughages, roots, and tubers were cut up into pea-sized pieces and were fed fresh in quantities estimated to furnish dry matter equivalent to the 40 gm. of alfalfa hay with which they were given.

Aliquot samples were taken at the time of each feeding and were kept in pyrex dishes at 50° C. in a Freas oven. At the end of an experiment the oven-dry samples were allowed to come to moisture equilibrium with the air. They were then weighed, ground in a Wiley mill, and retained for analysis, sealed in glass bottles.

Each of the concentrates was fed with an equivalent weight of alfalfa hay. Cottonseed cake was fed also with soybean hay and with vetch hay. The reasons for this will be given later.

Corn (maize) was fed coarsely cracked; all other grains were fed whole.

The oil cakes were fed in pea size, since rabbits do not like pulverized dry feeds.

Milk was fed in liquid form to the extent of 300 gm. daily with 40 gm. of alfalfa hay. No difficulty was encountered in getting the rabbits to drink 150 gm. of milk at each feeding.

The quantity of concentrate fed was usually 40 gm. daily, in addition to the 40 gm. of alfalfa hay. This combination provided nutriment somewhat in excess of the maintenance requirement.

The feed was given twice daily in equal portions and weighed immediately prior to feeding. Aliquot portions of the concentrates were saved each time the feeding stuffs were weighed, and the composite samples representing the collection period were ground and sealed up in the same way as were the roughage samples.

In general, three rabbits were used for each treatment. That this number was sufficient to establish valid digestion coefficients for the group was determined from experiments in which 10 or more trials were made.

COLLECTION AND TREATMENT OF FECES

The feces were usually collected over a period of 10 days. In certain cases when the supply of feed was insufficient or the animals

showed signs of malaise, the collection periods were curtailed to 7 days. Collection was always preceded by a preliminary feeding period of 5 to 7 days, usually the latter. The preliminary feeding was deemed to be sufficiently long to establish equilibrium between the feeding treatment and any effects of coprophagy that might have existed, since the quantity of feed eaten daily in two equal portions was constant and the daily weights of the feces were consistent after a few days.

Feces were collected daily throughout the experimental periods. The fresh feces were kept overnight at 50° C. in a ventilated Freas oven, from which they were transferred to open jars and left exposed to the air until the end of the collection period. They were then weighed each day during several days, to assure equilibrium with the air, and were then ground in a Wiley mill. The ground samples were kept in sealed bottles for analysis.

METHODS OF ANALYSIS

The feeds and feces were subjected to the conventional analyses for moisture, nitrogen, ether extract, crude fiber, ash, and energy, with nitrogen-free extract computed, as usual, by difference.

Moisture was determined as the loss in weight of a 2-gm. sample when heated overnight at 80° C. in a Freas oven and thereafter kept in a vacuum desiccator containing sulfuric acid until no further loss in weight occurred. Constant weight was obtained after 2 days in the vacuum desiccator. This procedure was found to be quite as accurate as drying to constant weight entirely in the vacuum desiccator, which would have required about 3 weeks.

Nitrogen was determined by the boric acid modification of the Kjeldahl method. Copper sulfate was used as the catalyst in the digestion, and methyl red as the indicator in the titration.

Ether extract was determined by extracting the dried samples employed in the moisture determination for 48 hours in Soxhlet extractors.

Crude fiber was determined on the residue from the ether extraction by the official method of the Association of Official Agricultural Chemists.

Ash was determined as the ignition residue from a 3- to 5-gm. sample which had been brought up slowly, overnight, to a temperature of 550° C. in a muffle furnace under rheostat control.

Energy was determined by means of an adiabatic Emerson bomb calorimeter.

RESULTS

DIGESTIBILITY AND DIGESTIBLE NUTRIENTS OF FEEDING STUFFS

The composition and the digestibility of the feeding stuffs, and the digestible nutrients contained therein, are presented in tables 1, 2, and 3, respectively. Supplementary tables 4, 5, 6, and 7 give the results of the similar work by Weiske (*6, 7, 8*), Von Knieriem (*3, 4*), and Brüggemann (*1*). The feeding stuffs on which results are reported both in the previously published studies referred to and in the investigation now presented are the following: Clover, timothy and vetch hays, barley, linseed cake, milk, oats, rye, and wheat. In general, the digestion coefficients given in the earlier and in the present work are in essential agreement.

A comparison of the results obtained for dry roughages with rabbits with those reported by Morrison (*2*) for similar feeds with ruminants shows, respectively, the following average digestibility: Protein, 65 and 60 percent; ether extract, 48 and 55; crude fiber, 17 and 53; and nitrogen-free extract, 58 and 65 percent.

TABLE 1.—*Composition of feeding stuffs supplied rabbits in this investigation*

Feeding stuff and source	Water	Crude protein	Ether extract	Carbohydrates		Ash	Energy per gram
				Crude fiber	Nitrogen-free extract		
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Calories*
Dry roughages:							
Alfalfa hay (Pennsylvania)	11.4	17.9	1.5	30.9	32.4	5.9	4.12
Alfalfa hay, western, 1938, (California)	6.5	20.4	2.2	20.4	42.1	8.4	4.20
Alfalfa hay, western, 1939, (California)	10.1	27.2	2.9	21.6	28.8	9.4	4.03
Bluegrass (California)	9.6	26.1	4.3	18.9	31.3	9.8	4.13
Clover hay (Pennsylvania)	12.5	12.9	2.7	24.7	41.2	6.0	3.91
Kale (California)	10.6	23.1	3.7	11.9	33.3	17.4	3.32
Kudzu hay (Alabama)	7.2	10.9	1.5	42.2	33.2	5.0	4.22
Lespedeza, common (Alabama)	10.5	14.8	2.8	27.7	39.0	5.2	4.29
Lespedza sericea (Alabama))	7.9	11.4	1.6	29.7	44.4	5.0	4.29
Lespedza sericea (Mississippi)	11.4	9.4	2.1	32.4	39.8	4.9	4.16
Melilotus hay (California)	10.6	21.1	2.9	22.6	33.0	9.8	3.99
Milo hay, green (California)	10.0	19.3	3.7	21.6	34.2	11.2	3.87
Oat hay, green (Pennsylvania)	6.8	7.1	3.1	37.4	38.3	7.3	4.10
Peanut hay (Alabama)	7.9	9.7	2.6	37.7	37.5	4.6	4.14
Soybean hay (Alabama)	10.4	10.6	1.4	38.0	33.2	6.4	3.95
Soybean hay, green (Pennsylvania)	7.5	14.5	1.9	28.6	37.5	10.0	3.94
Sudan grass (California)	11.0	15.8	3.7	20.2	40.5	8.8	3.88
Timothy hay (Pennsylvania)	10.1	5.9	1.8	30.7	47.1	4.4	4.07
Vetch hay (California)	10.3	19.2	3.1	24.5	35.3	7.6	3.96
Wheat hay, green (Pennsylvania)	10.3	19.4	3.6	23.2	33.7	9.8	3.93
Green roughages, roots, tubers:							
Cabbage	91.5	1.7	.1	.9	5.1	.7	.34
Carrot	87.7	1.4	.1	1.2	8.7	.9	.48
Celery	94.4	.9	.1	.8	2.5	1.3	.19
Rutabaga	86.9	1.3	.1	1.2	9.8	.7	.52
Sweetpotato	58.1	1.8	.3	1.0	37.9	.9	1.73
Turnip	91.8	1.0	.1	1.1	5.2	.8	.32
Yam	68.6	1.4	.4	.9	27.7	1.0	1.29
Concentrates:							
Barley	*14.5*	*10.7*	*1.2*	*6.4*	*65.0*	*2.2*	*3.88*
Beet pulp	11.5	8.3	.3	21.8	55.5	2.6	3.83
Bread, dried	4.4	15.8	2.5	.3	73.5	3.5	4.23
Brewers' grains	5.7	28.7	5.8	16.2	40.6	3.0	4.93
Buckwheat	14.8	10.4	2.3	10.8	60.0	1.7	3.85
Corn	*14.0*	*10.6*	*3.6*	*2.1*	*68.9*	*1.4*	*3.98*
Cottonseed-oil cake	6.7	39.7	6.6	13.3	26.8	6.9	4.55
Linseed-oil cake	10.8	36.8	4.0	7.8	35.2	5.4	4.29
Milk	87.8	3.1	3.7	------	4.6	.8	.72
Milo maize	6.5	12.1	2.8	1.9	75.0	1.7	4.03
Oats	12.1	10.9	4.2	10.6	58.9	3.3	4.18
Peanut-oil cake	6.0	42.8	7.7	4.4	32.6	6.5	4.57
Rye	14.1	9.7	1.4	2.1	70.9	1.8	3.91
Sesame-oil cake	5.5	39.4	8.7	6.7	26.5	13.2	4.22
Soybean-oil cake	9.3	45.5	4.0	5.0	29.9	5.7	4.44
Soybeans, whole, Manchu	6.8	40.9	17.1	5.6	24.5	5.1	5.23
Wheat	14.9	8.9	1.3	2.2	70.9	1.8	3.79
Wheat bran	10.4	16.7	3.9	10.5	51.6	6.9	4.09
Mixed rations:							
Purina omolene	*12.1*	*12.4*	*2.7*	*7.8*	*60.6*	*4.4*	*3.98*
Purina rabbit chow	11.0	14.9	3.3	13.7	52.1	5.0	4.02

ABLE 2.—*Average digestibility of feeding stuffs supplied rabbits in this investigation*

Feeding stuff and source	Trials	Dry matter	Crude protein	Ether extract	Carbohydrates		Ash	Energy
					Crude fiber	Nitrogen-free extract		
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
ry roughages:								
Alfalfa hay (Pennsylvania)	19	48.1	72.3	16.0	18.2	63.3	62.4	45.9
Alfalfa hay, western, 1938 (California)	3	67.5	80.7	20.0	29.8	81.9	67.4	64.6
Alfalfa hay, western, 1939 (California)	3	63.4	83.6	39.1	27.3	74.8	60.7	61.6
Bluegrass (California)	3	45.2	74.4	40.9	12.6	41.0	45.7	45.4
Clover hay (Pennsylvania)	3	52.8	62.8	61.8	19.7	67.2	64.3	49.3
Kale (California)	3	76.0	81.1	40.7	59.5	83.4	74.1	69.3
Kudzu hay (Alabama)	3	38.0	62.5	12.4	16.1	55.8	59.0	34.4
Lespedeza, common (Alabama)	3	44.7	66.7	45.9	10.6	59.3	53.1	43.0
Lespedeza sericea (Alabama)	3	31.5	28.7	54.3	7.3	46.5	41.4	28.6
Lespedeza sericea (Mississippi)	3	32.9	30.8	50.0	8.5	50.2	51.1	30.5
Melilotus hay (California)	3	56.9	79.0	49.2	6.5	73.4	72.4	53.6
Milo hay, green (California)	2	44.8	71.1	46.3	15.7	46.6	49.5	42.7
Oat hay, green (Pennsylvania)	1	29.1	61.0	53.8	10.4	35.5	50.0	26.9
Peanut hay (Alabama)	3	47.1	55.0	09.7	25.0	65.9	46.1	44.2
Soybean hay (Alabama)	1	41.3	67.1	31.8	16.6	58.9	54.6	38.1
Soybean hay, green (Pennsylvania)	3	52.3	69.6	9.0	24.0	71.7	43.6	49.6
Sudan grass (California)	3	53.8	68.1	49.4	26.6	64.1	44.9	52.3
Timothy hay (Pennsylvania)	3	36.5	46.6	44.2	10.7	51.0	45.3	34.0
Vetch hay (California)	3	56.2	78.3	62.8	11.0	72.4	68.5	52.6
Wheat hay, green (Pennsylvania)	3	50.5	77.6	50.3	22.0	53.2	55.7	49.3
een roughages, roots, tubers:								
Cabbage	3	101.3	98.6	83.3	88.2	102.9	94.1	100.3
Carrot	3	92.8	85.7	79.4	56.4	97.8	87.6	90.8
Celery	3	92.5	76.8	90.7	93.3	98.8	86.6	89.3
Rutabaga	3	99.3	89.4	83.3	103.6	100.0	96.0	97.6
Sweetpotato	2	92.6	43.8	75.6	94.3	94.4	103.7	90.4
Turnip	3	97.7	90.6	102.9	82.1	101.0	86.2	96.7
Yam	3	96.1	51.7	80.2	123.1	96.8	109.0	93.7
oncentrates:								
Barley	3	82.5	84.8	106.2	12.5	89.3	41.1	82.4
Beet pulp	3	82.1	48.3	−52.4	71.8	91.6	77.7	79.1
Bread, dried	3	100.2	95.2	98.5	-----	100.9	105.4	99.0
Brewers' grains	3	56.4	84.7	80.8	20.6	48.7	15.0	60.0
Buckwheat	3	78.7	72.2	95.2	16.5	89.3	89.2	76.1
Corn	10	91.3	84.2	93.1	145.8	91.9	44.8	92.0
Cottonseed-oil cake	4	66.0	83.5	98.6	30.7	56.3	33.6	70.4
Linseed-oil cake	3	77.3	86.4	98.8	20.5	81.1	34.6	79.4
Milk	3	92.3	99.6	95.3	-----	94.1	84.1	91.1
Milo maize	3	89.3	72.1	95.6	103.1	90.6	105.8	86.9
Oats	3	68.2	78.6	97.6	−2.4	78.7	20.7	69.6
Peanut-oil cake	3	91.4	91.2	101.1	48.9	96.2	69.2	92.3
Rye	3	90.5	79.1	82.1	53.6	93.0	86.2	89.7
Sesame-oil cake	3	81.6	90.9	101.3	73.2	84.4	30.2	90.3
Soybean-oil cake	4	89.7	89.7	96.1	51.9	95.5	66.8	90.0
Soybeans, whole, Manchu	3	88.8	87.9	95.5	85.3	90.8	62.9	89.4
Wheat	3	93.3	84.6	100.0	28.5	96.6	53.6	92.8
Wheat bran	3	62.6	83.0	77.2	24.3	65.3	33.0	64.3
ixed rations:								
Purina otmolene	6	74.9	76.3	87.2	17.0	82.8	52.7	74.6
Purina rabbit chow	5	68.1	76.3	81.0	18.2	78.9	58.8	67.2

TABLE 3.—*Dry-matter content and digestible nutrients of feeding stuffs suppli rabbits in this investigation*

Feeding stuff and source	Total dry matter	Digestible nutrients in—					
		Crude protein	Carbohydrates			Fat	Tot
			Crude fiber	Nitrogen-free extract	Total		
Dry roughages:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Perc*
Alfalfa hay (Pennsylvania)	88.6	12.9	5.6	20.5	26.1	0.5	39
Alfalfa hay, western, 1938 (California)	93.5	16.5	6.1	34.5	40.6	1.0	58
Alfalfa hay, western, 1939 (California)	89.9	22.7	5.9	21.5	27.4	2.5	52
Bluegrass (California)	90.4	19.4	2.4	12.8	15.2	4.0	38
Clover hay (Pennsylvania)	87.5	8.1	4.9	27.7	32.6	3.8	44
Kale (California)	89.4	18.7	7.1	27.8	34.9	3.4	57
Kudzu hay (Alabama)	92.8	6.8	6.8	18.5	25.3	.4	32
Lespedeza, common (Alabama)	89.5	9.9	2.9	23.1	26.0	2.9	38
Lespedeza sericea (Alabama)	92.1	3.3	2.2	20.6	22.8	2.0	28
Lespedeza sericea (Mississippi)	88.6	2.9	2.8	20.0	22.8	2.4	28
Melilotus hay (California)	89.4	16.7	1.5	24.2	25.7	3.2	45
Milo hay, green (California)	90.0	13.7	3.4	15.9	19.3	3.8	36
Oat hay, green (Pennsylvania)	93.2	4.3	3.9	13.6	17.5	3.8	25
Peanut hay (Alabama)	92.1	5.3	9.4	24.7	34.1	4.1	43
Soybean hay (Alabama)	89.6	7.1	6.3	19.6	25.9	1.0	34
Soybean hay, green (Pennsylvania)	*92.5*	*10.1*	*6.9*	*26.9*	*33.8*	*.4*	*44*
Sudan grass (California)	89.0	10.8	5.4	26.0	31.4	4.1	46
Timothy hay (Pennsylvania)	89.9	2.7	3.3	24.0	27.3	1.8	31
Vetch hay (California)	89.7	15.0	2.7	25.6	28.3	4.4	47
Wheat hay, green (Pennsylvania)	89.7	15.1	5.1	17.9	23.0	4.1	42
Green roughages, roots, and tubers:							
Cabbage	8.5	1.7	.8	5.1	5.9	.2	7
Carrot	12.3	1.2	.7	8.5	9.2	.2	10
Celery	*5.8*	*.7*	*.7*	*2.5*	*3.2*	*.2*	*4*
Rutabaga	13.1	1.2	1.2	9.8	11.0	.2	12
Sweetpotato	41.9	.8	.9	35.8	36.7	.5	38
Turnip	8.2	.9	.9	5.2	6.1	.2	7
Yam	31.4	.7	.9	26.8	27.7	.7	28
Concentrates:							
Barley	85.5	9.1	.8	58.0	58.8	2.7	70
Beet pulp	88.5	4.0	15.7	50.8	66.5	.0	70
Bread, dried	*95.6*	*15.0*	*.0*	*73.5*	*73.5*	*5.5*	*94*
Brewers' grains	94.3	24.3	3.3	19.8	23.1	10.6	57
Buckwheat	85.2	7.5	1.8	53.6	55.4	4.9	67
Corn	86.0	8.4	2.1	63.3	65.4	7.5	81
Cottonseed-oil cake	93.3	33.1	4.1	15.1	19.2	14.6	66
Linseed-oil cake	89.2	31.8	1.6	28.5	30.1	8.9	70
Milk	12.2	3.1	------	4.3	4.3	7.9	15
Milo maize	93.5	8.7	1.9	68.0	69.9	6.0	84
Oats	*87.9*	*8.8*	*.9*	*46.4*	*48.4*	*8.2*	*84*
Peanut-oil cake	94.0	39.0	2.2	31.4	33.6	17.3	89
Rye	85.9	7.7	1.1	65.9	67.0	2.6	77
Sesame-oil cake	94.5	35.8	4.9	22.4	27.3	19.6	82
Soybean-oil cake	90.7	40.8	2.6	28.6	31.2	9.9	81
Soybeans, whole, Manchu	93.2	36.0	4.8	22.2	27.0	30.7	94
Wheat	85.1	7.5	.6	68.5	69.1	2.9	79
Wheat bran	89.6	13.9	2.6	33.7	36.3	6.8	57
Mixed rations:							
Purina omolene	87.9	9.5	1.3	50.2	51.5	5.3	66
Purina rabbit chow	89.0	11.4	2.5	41.1	43.6	6.0	61

ABLE 4.—*Composition of feeding stuffs supplied rabbits in investigations of Von Knieriem and Weiske*

Feeding stuff	Water	Crude protein	Ether extract	Carbohydrates: Crude fiber	Carbohydrates: Nitrogen-free extract	Ash	Reference
y roughages:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Barbarea vulgaris hay	13. 7	14. 3	3. 1	28. 2	34. 1	6. 6	Von Knieriem (*3, 4*).
Clover hay, white	13. 4	16. 0	3. 8	17. 2	40. 5	9. 1	Do.
Clover hay, red	13. 3	13. 5	4. 0	24. 3	39. 3	5. 6	Do.
Darnel grass hay	8. 3	6. 3	2. 7	36. 0	42. 4	4. 3	Do.
Geum rivale hay	12. 5	8. 9	3. 4	23. 4	43. 3	8. 5	Do.
Kidney-vetch hay	11. 8	10. 3	3. 2	28. 9	39. 5	6. 3	Do.
Orchard grass, rank	11. 8	10. 2	2. 8	28. 1	41. 0	6. 1	Do.
Orchard grass, spare	11. 4	6. 8	2. 2	29. 2	43. 5	6. 9	Do.
Timothy hay	10. 3	6. 5	1. 6	36. 9	39. 6	5. 1	Do.
Vetch hay	15. 3	15. 5	2. 8	23. 4	32. 3	10. 7	Do.
oncentrates:							
Barley	6. 9	11. 3	2. 0	4. 2	71. 9	3. 7	Weiske (*8*).
Coconut cake	12. 0	21. 8	13. 5	14. 4	33. 0	5. 3	Von Knieriem (*3, 4*).
Hempseed cake	13. 7	26. 1	9. 2	29. 9	12. 6	8. 5	Do.
Linseed cake	11. 9	30. 1	9. 0	9. 2	33. 2	6. 6	Do.
Milk	90. 7	3. 5	. 5	--------	4. 5	. 8	Do.
Oats	6. 5	11. 1	5. 5	9. 3	64. 7	2. 9	Weiske (*6, 7*).
Palm-kernel cake	10. 5	16. 9	12. 0	17. 4	39. 0	4. 2	Von Knieriem (*3, 4*).
Rapeseed cake	10. 6	29. 9	8. 5	11. 9	32. 1	7. 0	Do.
Rye	6. 0	12. 0	1. 7	2. 1	76. 3	1. 9	Weiske (*8*).
Sunflower cake	9. 5	30. 7	9. 5	19. 4	25. 8	5. 1	Von Knieriem (*3, 4*).

ABLE 5.—*Digestibility of feeding stuffs supplied rabbits in investigations of Von Knieriem and Weiske*

Feeding stuffs	Dry matter	Crude protein	Ether extract	Carbohydrates: Crude fiber	Carbohydrates: Nitrogen-free extract	Ash	Reference
y roughages:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Barbarea vulgaris hay	55. 3	77. 9	62. 4	25. 9	66. 3	72. 3	Von Knieriem (*3, 4*).
Clover hay, white	72. 7	68. 2	50. 9	57. 4	83. 1	72. 4	Do.
Clover hay, red	55. 1	64. 4	75. 3	26. 5	68. 2	53. 6	Do.
Darnel grass hay	35. 4	54. 2	57. 2	12. 5	51. 8	22. 7	Do.
Geum rivale hay	55. 2	32. 9	62. 0	25. 6	73. 1	66. 5	Do.
Kidney-vetch hay	55. 9	65. 8	60. 1	27. 1	73. 5	59. 6	Do.
Orchard grass, rank	47. 6	76. 0	64. 5	15. 2	58. 5	68. 5	Do.
Orchard grass, spare	44. 2	71. 8	63. 1	12. 4	59. 4	55. 9	Do.
Timothy hay	35. 6	56. 7	55. 0	18. 5	48. 2	27. 4	Do.
Vetch hay	56. 7	71. 3	58. 0	29. 9	69. 2	56. 3	Do.
oncentrates:							
Barley	84. 0	67. 7	86. 3	25. 1	91. 2	51. 2	Weiske (*8*).
Coconut cake	94. 4	95. 7	99. 1	89. 1	95. 2	86. 6	Von Knieriem (*3, 4*).
Hempseed cake	--------	65. 2	90. 1	29. 3	8. 2	11. 3	Do.
Linseed cake	--------	86. 0	93. 4	28. 1	76. 0	38. 7	Do.
Milk	--------	97. 7	91. 7	--------	97. 8	80. 0	Do.
Oats	73. 7	80. 2	93. 8	21. 6	79. 5	46. 4	Weiske (*8*).
Palm-kernel cake	--------	92. 8	97. 7	64. 0	77. 9	--------	Von Knieriem (*3, 4*).
Rapeseed cake	--------	78. 9	85. 4	39. 8	73. 2	--------	Do.
Rye	84. 4	63. 0	76. 3	18. 5	91. 2	34. 4	Weiske (*8*).
Sunflower cake	--------	85. 7	79. 1	13. 7	45. 0	--------	Von Knieriem (*3, 4*).

TABLE 6.—*Dry-matter content and digestible nutrients of feeding stuffs suppli rabbits as computed from data of Von Knieriem and Weiske*

Feeding stuff	Total dry matter	Digestible nutrients in—						Reference
		Crude protein	Carbohydrates			Fat	Total	
			Crude fiber	Nitrogen-free extract	Total			
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Dry roughages:								
Barbarea vulgaris hay	86.3	11.1	7.3	22.6	29.9	4.4	45.4	Von Knieriem (3,
Clover hay, white	86.6	10.9	9.9	33.7	43.6	4.4	58.9	Do.
Clover hay, red	86.7	8.7	6.4	26.8	33.2	6.8	48.7	Do.
Darnel grass hay	91.7	4.0	4.5	22.0	26.5	3.5	34.0	Do.
Geum rivale hay	87.5	2.9	6.0	31.7	37.7	4.7	45.3	Do.
Kidney-vetch hay	88.2	6.8	7.8	29.0	36.8	4.3	47.9	Do.
Orchard grass rank	88.2	7.8	4.3	24.0	28.3	4.1	40.2	Do.
Orchard grass, spare	88.6	4.9	3.6	25.8	29.4	3.1	37.4	Do.
Timothy hay	89.7	3.7	6.8	19.1	25.9	2.0	31.6	Do.
Vetch hay	84.7	11.1	7.0	22.4	29.4	3.7	44.2	Do.
Concentrates:								
Barley	93.1	7.7	1.1	65.6	66.7	3.9	78.3	Weiske (*8*).
Coconut cake	89.0	20.9	12.8	31.4	44.2	30.1	95.2	Von Knieriem (3,
Hempseed cake	86.3	17.0	8.8	1.0	9.8	18.7	45.5	Do.
Linseed cake	88.1	25.9	2.6	25.2	27.8	18.9	72.6	Do.
Milk	9.3	3.4	-----	4.5	4.5	1.0	8.9	Do.
Oats	93.5	8.9	2.0	51.4	53.4	11.6	73.9	Weiske (*8*).
Palm-kernel cake	*89.5*	*15.7*	*11.1*	*30.4*	*41.5*	*26.4*	*83.6*	Von Knieriem (3,
Rapeseed cake	89.4	23.6	4.7	23.5	28.2	16.3	68.1	Do.
Rye	94.0	7.6	1.4	69.6	71.0	2.9	81.5	Weiske (*8*).
Sunflower cake	90.5	26.3	2.7	11.6	14.3	16.9	57.5	Von Knieriem (3,

TABLE 7.—*Digestibility and digestible nutrients of feeding stuffs supplied rabb as computed from data of Brüggemann (1)*

Feeding stuff	Digestibility of—					Digestible nutrie	
	Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen-free extract	Protein	Tota
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Perce*
Meadow hay	47.2	60.1	43.1	18.5	64.5	5.2	3
Lucerne hay	61.3	75.7	15.9	38.6	72.6	16.6	4
Stinging nettle hay	63.0	95.6	31.6	42.4	78.9	20.8	5
Green lucerne	81.5	89.1	70.8	66.0	88.0	5.6	1
Green sweet lupine	78.9	91.1	73.2	56.5	84.1	3.3	[illegible]
Ensilaged maize	55.0	79.4	96.1	24.2	68.8	1.3	1
Oats	63.8	79.0	90.3	18.0	72.2	10.3	5
Barley	89.6	78.0	90.2	72.2	93.3	9.7	7
Wheat	*94.0*	*81.0*	*91.8*	*90.5*	*97.0*	*10.9*	*8*
Beet roots	96.6	66.4	-------	100.0	95.7	.3	1
Steamed potatoes	98.3	58.3	100.0	100.0	99.1	1.3	2

It is noticeable from this comparison that the digestibility of tl crude fiber of dry roughages by rabbits is remarkably low. Th observation is not new, having been made by Von Knieriem as earl as 1898 and recently confirmed by Brüggemann (*1*) and Watson an Godden (*5*). The latter workers found the total digestibility, as we as that of crude fiber, of pasture herbage, consisting of mixed grass with some clover, to be less with rabbits than with sheep. They su gested that the lower digestibility of crude protein, ether extrac and nitrogen-free extract might well be due to sweeping-out or occl sion effects of the indigestible fiber. Such effects are not evide from the present data, since the digestibility of the constituents oth than crude fiber compares fairly well with that for ruminants.

From the data reported by Morrison (*2*) for horses and swine it appears that the capacity of rabbits to digest crude fiber is less than that of horses and nearly equivalent to that of swine.

Since the digestibility of true fat by rabbits is almost complete, as evidenced by the digestibility of the ether extracts from the whole grains, seeds, and oil cakes, it seems quite justifiable to suggest that the percentage digestibility of the ether extracts from the dry roughages furnishes a rough measure of the proportion of the fatty acid esters to the nonlipide constituents of the ether extracts.

Among the better roughages for rabbits, judging by the digestible nutrients they furnish, are dried kale, alfalfa, vetch, and clover hay green soybean and green wheat hay, and Sudan grass. Peanut hay might be included in this list, but the lot of hay employed in the present work contained some nuts, which may have accounted for a considerable portion of the digestible nutrients.

The poorest of the dry roughages was definitely oat hay. Not only was it unpalatable but the weight of the feces from the hay almost equaled that of the hay itself. One rabbit died, apparently of undernutrition, after subsisting on 60 gm. of oat hay daily for 9 days. Anotber rabbit, subsisting on 80 gm. of oat hay daily, lost 560 gm. or 30 percent of its original weight, in 2 weeks.

A noticeable difference in palatability was evident between the *Lespedeza sericea* from Mississippi and that from Alabama. The former had a pronounced odor of tea and was much less palatable than the sample from Alabama. The digestibility of tbe two samples however, was not considerably different.

All the constituents of the green roughages, roots, and tubers were found highly digestible by rabbits. Even the crude fiber of these feeding stuffs was highly digestible. It is obvious, however, that the method of determination of crude fiber is not sufficiently definitive to allow interpretation of the high digestibility of crude fiber in these feeding stuffs as compared with its low digestibility in the dry roughages. It appears that the chief virtue of the green roughages and roots as feeding stuffs for rabbits is in their accessory food factors rather than in the quantities of digestible nutrients which they supply

In regard to the concentrates—those which furnished digestible nutrieuts in excess of 80 percent of their weight are listed in order as follows: Soybeans, dried bread, peanut-oil cake, milo maize, sesame oil cake, and corn. Wheat, rye, linseed-oil cake, barley, and dried beet pulp furnish digestible nutrients ranging from 70 to 80 percent of their weight. The constituents of milk are highly digestible by rabbits, but this feeding stuff is doubtless more valuable in furnishing accessory food factors than quantities of digestible nutrients. Soybean-oil cake, peanut-oil cake, soybeans, sesame-oil cake, linseed-oil cake, and brewer's grains may be listed as notable sources of protein for rabbits.

TOXICITY OF COTTONSEED-OIL CAKE FOR RABBITS

There seem to be no published observations on the toxicity of cottonseed-oil cake for rabbits. In the early part of this work cottonseed-oil cake was offered in pellet form, with an equal weight of alfalfa hay, to three rabbits. Two of these rabbits refused to eat the cottonseed pellets after the second day. The third rabbit continued to eat the cottonseed for 9 days before refusing. This rabbit

ceased producing feces and lost all appetite for any kind of feed offered. By the eleventh day its abdomen was hard and bloated, and it died on the thirteenth day after cottonseed-oil cake had first been eaten. Post-mortem examination indicated practically complete intestinal stasis. The contents of the caecum and lower gut were hard-packed and dry.

Attempts to feed other rabbits on cottonseed pellets and alfalfa hay ended with their rejection of the cottonseed after 2 or 3 days. No obvious ill effects appeared in these rabbits.

At a later date three rabbits were offered pellets of cottonseed-oil cake with vetch hay. They refused to eat the cottonseed after 6 days, showing signs of constipation. Two of these rabbits recovered after being fed cabbage and a commercial rabbit ration for several days. The third rabbit continued to fail; and, because of the advancing symptoms of anorexia, constipation, abdominal rigidity, etc., it was killed for autopsy. Among the overt symptoms, intestinal stasis was again marked. The lungs, kidney, heart, and liver appeared normal.

The feeding of cottonseed-cake pellets with soybean hay (green, *dried, from Pennsylvania) was unique in that the rabbits continued* to eat the cottonseed over a much longer period than with the other roughages. One rabbit exhibited no ill effects after receiving 40 gm. of cottonseed cake with 40 gm. of soybean hay daily for 16 days. None of the unfavorable symptoms observed previously in other rabbits appeared in this one; but 4 days after being taken off the cottonseed diet and put onto a commercial rabbit ration the rabbit died. The second of these rabbits ate the cottonseed with soybean hay for 15 days before exhibiting signs of constipation and failure of appetite. This rabbit continued to fail and was killed on the eighteenth day after initiation of the cottonseed feeding. The third rabbit in this group rejected the cottonseed-soybean hay diet after 14 days, lingered for 7 days by nibbling at offerings of cabbage and commercial rabbit ration, but died on the twenty-second day after the initiation of the cottonseed feeding.

The cottonseed cake fed was made from seed grown in the San Joaquin Valley, Calif., in 1937.

No study was made of the cause of the observed toxicity of the cottonseed-oil cake.

SUMMARY

The composition, digestibility, and digestible nutrients of 47 feeding stuffs, including dry roughages, green roughages, roots, tubers, concentrates, and commercial mixed feeds, for rabbits, are reported. The greater part of the determinations were made in triplicate.

Concentrates were found to be well digested, and, with the exception of crude fiber, roughages were nearly as well digested by rabbits as by domestic animals.

Kale, alfalfa, vetch, clover, soybean, wheat, and Sudan grass were found to be among the better roughages for rabbits.

The poorest roughages studied were bluegrass and oat hay.

When fed as the sole concentrate, with the same quantity of roughage, cottonseed meal was found to be toxic to rabbits, the most prominent effects being intestinal stasis and loss of appetite.

esults of previously published work are tabulated and all available concerning the utilization of feeding stuffs by rabbits are thus ight together.

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CANE GALL OF BRAMBLES CAUSED BY PHYTOMONAS RUBI N. SP.[1]

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INTRODUCTION

Because of a similarity in gross symptoms, cane gall has often been confused with crown gall caused by *Phytomonas tumefaciens* (Sm. and Town.) Bergey *et al.*, although its identity was apparently established by Banfield (*2, 4, 18*).[2] Pinckard (*13*), in a comparative study of the physiology of cell-stimulating bacteria, demonstrated rather conclusively specific differences between these two organisms and provided a basis for describing the cane gall organism. The present paper summarizes the results of observations made on this disease and its causal organism in New York State during the past 7 years and brings together other pertinent facts from the literature to assist in describing the causal organism.

Cane gall is undoubtedly an old disease since over 40 years ago Bailey (*1*) reported in New York a so-called cane-knot disease of blackberries and illustrated typical cane gall symptoms. He states: "It is apparently not common, but it must be widespread for I have had specimens from as far west as Wisconsin." Shortly thereafter a similar disease was reported from Europe (*17, p. 606, 19, 20*). That cane gall now occurs widely in the raspberry-growing sections of the United States (Ohio, Indiana, Michigan, Illinois, and Oregon) has been indicated by Banfield (*4, 18*). The writer found it in New York in 1932 and Zundel (*21*) has noted its presence in Pennsylvania.

SYMPTOMS

Symptoms appear on the fruiting canes of *Rubus* spp. in late May or June as small spherical protuberances or elongate ridges of white granular gall tissue (fig. 1). The small whitish eruptions rapidly increase in size and number and may completely cover sections of the cane surface, being most abundant on the lower part of the cane but appearing also on the upper part and even on the small terminal branches. After several weeks the whitish gall tissue turns brown and begins to disintegrate near the soil surface (fig. 2). The enlargement of the galls frequently causes the stems to split open and the canes to dry out. These injured canes produce only small seedy berries. Growers refer to the condition as "beading," "coraling," or "knotting."

Under severe disease conditions one New York grower wrote Bailey (*1*), that the disease "progresses rapidly, as the fruit grows, and when the fruit is about two-thirds grown the leaves begin to wither,

[1] Received for publication March 20, 1940.
[2] Italic numbers in parentheses refer to Literature Cited, p. 695.

the cane dries up, and the berries ripen. On very badly disease the berries wither and dry up." The course of the disease as o above was very similar to that observed on the Sodus var purple raspberry in the writer's garden in 1939.

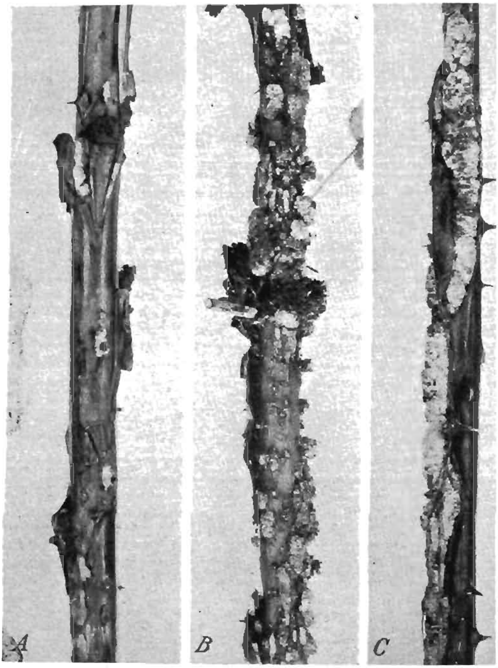

FIGURE 1.—Stages in cane gall development on black raspberry. The firs toms appear (*A*) on the fruiting canes in May as spheres or ridges o granular tissue. The galls increase in number and size and during Ju may appear singly or in rows (*B*) or in more or less elongate ridges (*C*). graphs furnished by W. M. Banfield).

According to Banfield, cane galls are seldom found on the of black raspberry, the plant principally used in his studies, the roots or canes of red raspberry, and rarely, if ever, on blacl berry canes during the first year of their growth. Bailey (*1*) that the disease, "probably attacks the growing shoots, althou

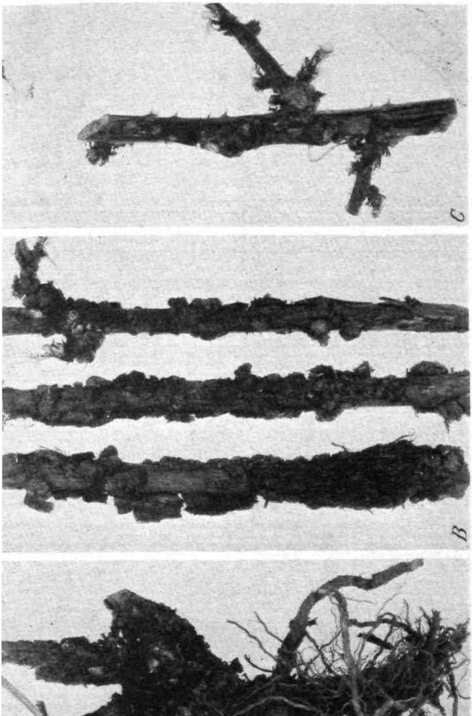

FIGURE 2.—Advanced stage of cane gall on a Plum Farmer black raspberry plant collected in the field in July. Practically all of the galls are discolored and in an advanced stage of deterioration and there is little new cane growth: *A*, Plant showing disintegration of the crown region and sparseness of current season cane growth therefrom; *B*, abundance of galls along the fruiting canes (sometimes these appear in rows and sometimes in indiscriminate order); *C*, galls that have developed on the side branches well up on the plant.

not apparent until the following year, when the grower, noticing that the leaves are yellow and the fruit not filling, examines the canes and finds these knots upon them."

ISOLATION STUDIES

The cane gall organism was readily isolated from fresh young galls by using poured plates of such media as potato-dextrose or potato-mannitol agar.[3] Banfield (4) induced cane galls on black raspberry under aseptic conditions, and when isolations were attempted 3 weeks later only cane gall bacteria appeared on the plates. Numerous isolations by the writer from young galls of about the same age, but produced under exposed conditions, generally gave cultures relatively free from contaminants. However, when isolations were attempted from older galls and from soil, serious difficulty was encountered with contaminants, even in the former case, although the greatest care was exercised in removing the outer gall tissue before taking samples.

To discover a more suitable isolation medium, a comparative study was made involving, in addition to the common laboratory media, several selective media (7, 11, 12) of which the aniline blue medium of Hendrickson et al. proved fairly satisfactory. The bacterial colonies resulting from tissue and soil isolations came up in aniline blue agar plates largely free from contaminants.

Whenever discoloration became pronounced in the cane galls, which was a rather common occurrence in the field in July, it was no longer possible to recover cane gall bacteria from them by any method tried.

Before pathogenicity tests were carried out, the parent cultures were purified by making single-cell isolations (10). Five single cells were isolated from each of the original parent cultures obtained from two black and one purple raspberry planting in New York. Growth resulted from two cells isolated from culture 1, five from culture 2, and three from culture 3. These 13 isolates (counting both parents and progeny) appeared identical in culture and were employed in making subsequent studies.

PATHOGENICITY

Galls were readily induced by inoculation of the organism into fruiting canes, current season cane growth and petioles, and roots. Figure 3, *A*, illustrates an early gall development on a current season cane and petiole stimulated by needle-puncture inoculation. A later stage produced in the same manner is shown in figure 3, *B*. A high incidence of infection ranging from 80 to 100 percent usually resulted from needle-puncture inoculations into current-season canes. Figure 3, *C*, illustrates an advanced stage of gall development on new growth, the radial extension of the gall being over 1 inch. Brown patches over the surface indicate that decomposition is taking place.

Small galls were produced on the roots of plants when the bacteria were introduced into the soil either directly or by means of water washing the bacteria off galls above ground. Wounds for infection courts

[3] The ingredients for 1 liter of potato-dextrose agar consisted of 200 gm. of potato (skins removed), 17 gm. of agar, 10 or 20 gm. of dextrose, and distilled water. The same ingredients were used in potato-mannitol agar except for the substitution of 5 gm. of mannitol for the dextrose and the addition of a small pinch of finely powdered calcium carbonate.

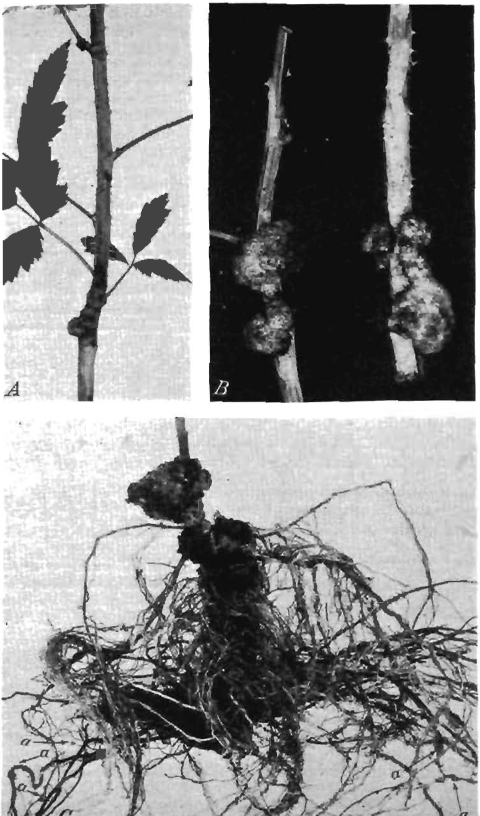

FIGURE 3.—Results of needle-puncture inoculations with the cane gall organism on Plum Farmer black raspberry plants: *A*, Gall symptoms on a petiole and stem of new cane growth 4 weeks after inolcuation; *B*, cane galls on current season stems 2 months after inoculation, with discoloration beginning to set in; *C*, cane galls on current-season growth about 11 weeks after inoculation. These galls measure over 1 inch in radial extension and the surface discoloration indicates a rapid state of deterioration. Small root galls (*a*) are also present

were produced by probing among the roots with a sharp tool. Several small galls of this character may be seen in figure 3, *C*, *a*.

When inoculations were made early in the season into fruiting canes, the incidence of infection was lower than when they were made into current-season growth. The reason for this was not ascertained. However, when infections were obtained there was a tendency for the galls to form ridges upward on the stem, the phenomenon so characteristic of infections occurring in the field.

HOST RANGE

Cane gall has been found in New York commercial plantings on black raspberries, purple raspberries, and blackberries, and when inoculated with the cane gall organism these plants and red raspberries also have been found susceptible to infection. On black raspberry canes 25 inoculations induced 25 galls with an average radial extension of 22 mm. after 6 weeks. Similarly on purple raspberry, red raspberry, and blackberry canes, 25 inoculations induced 25, 18, and 25 galls, respectively, with average radial extensions of 20, 4, and 18 mm. These results confirm a report (*18*) from Wisconsin for black raspberries. Although studies on host range are incomplete, it probably deserves mention that none of the plants thus far tested except the brambles have been found susceptible to infection. Pinckard (*13*) also failed to secure infection on tomato, beet, oleander, and olive.

LIFE HISTORY

The life history of the cane gall organism in relation to pathogenesis has received considerable attention especially from Banfield (*4*), but several details still remain to be determined.

The organism seems to enter the plant only through wounds or other injuries, for applications of bacteria to the surfaces of uninjured petioles, stems, and roots have always given negative results. The exact mode of entry in the field is unknown; however, root-feeding insects (*3*) or other insects that injure the plants, pruning wounds, and injuries to the underground parts produced in cultural operations, all would seem to afford adequate opportunity for bacterial invasion.

Banfield (*3*), in studying crown gall on red raspberry, observed that infections occurred only through injuries. A further significant fact observed was that injured tissue on the underground parts of red raspberry remain infection courts for as long as 7 weeks after injury. This slowness of wounds to heal favors the likelihood of invasion by all forms of wound pathogens, including the cane gall organism. Of perhaps equal significance is the delayed incubation period. This has been established for crown gall (*5*), but undoubtedly plays as significant a role in cane gall.

The incubation period (latent period of infection) based on inoculations during the summer months (June to September) was never less than about 2½ weeks and averaged nearer 3 weeks. This interval is somewhat longer than for the crown gall organism on raspberry as reported by Banfield (*3*). From two to four plants were inoculated at monthly intervals by needle punctures, each plant receiving an average of five inoculations into the growing canes. By judicious pruning, new growth was available each month. Inoculations were not attempted later than September, but for April and May the incubation periods averaged approximately 6 and 4 weeks.

The location of the casual organism in cane galls on black raspberry was thoroughly studied by Banfield (*4*), who found the bacteria principally between the cell walls but ramifying throughout all regions of the gall in the form of zoogloeal strands which dissolved away the middle lamella of the cell walls for channels. The gall initials found beneath the phelloderm of fruiting canes in early spring appear to be induced by hyperplasia [4] of the pericycle and phloem ray cells. From the time of the first microscopic appearance of the gall to its maturity, bacterial pockets are of common occurrence in the meristematic areas. Apparently they result from successive lysis and collapse of cells in contact with the bacterial strands; the cells just beyond the bacterial strands divide and those farther removed are not stimulated. As the galls near maturity, cell division becomes progressively more feeble and tissue degeneration more general. It is an interesting fact that the cell walls which are composed largely of cellulose appear not to be dissolved by the bacteria.

The bacteria may be discharged (*4*) from the cavities or pockets in the gall before disintegration sets in. Discharge takes place by way of intercellular channels to the surface which are presumed to have been occupied by bacterial strands.

The cane gall organism was found only in gall tissue when current season stems were inoculated at two widely separated points. Galls 2½ months old produced near the apex or the base of current season canes when they were about 12 inches long were employed. In one case isolations were attempted from the fresh galls and from the apparently healthy stem between these galls cut into four 3-inch pieces. Bacteria were obtained only from the gall tissue. In another case, the galls and stem pieces were immersed in calcium hypochlorite solution [5] for 20 minutes to reduce surface contaminants. Subsequently the galls were passed through sterile distilled water seven times, changing at hourly intervals. Platings were made from the washings, employing potato-mannitol agar and aniline blue selective medium. The results indicate that the bacteria were limited to the immediate gall region, that they had not migrated in current season canes, and that they were freely given off at the gall surface when immersed in water. Only one washing, that immediately following immersion in calcium hypochlorite solution, failed to yield bacteria in abundance from the uninjured galls, and this was undoubtedly due to the residual effects of the disinfectant.

In a similar experiment on fruiting canes the bacteria were obtained from the galls and also from the stem tissue for a short distance (up to 10 mm.) immediately above the galls, a fact which suggests that the fruiting canes must differ in some respects from current-season canes since they afford passage for the bacteria.

Longevity experiments seem to indicate that the period of survival of the cane gall bacterium in soil is much shorter than the periods of a year or more found earlier for the hairy root (*8*) and crown gall (*3*) organisms. Steamed and unsteamed soil were artificially infested with cane gall bacteria and stored either out of doors or in the greenhouse in early October. Isolations were attempted at monthly intervals, the isolation technique of Hildebrand (*8*) being used. The soil samples

[4] Butler (*6*), who examined histologically cane galls on black raspberry received from Wisconsin, mentions the presence of both hypertrophy and "hyperplasy" in the older gall tissue.

[5] Consists of 10 gm. of calcium hypochlorite mixed with 140 cc. of distilled water, filtered, and used fresh at full strength.

consisted of duplicate ½-cubic-foot lots of moderately rich clay loam placed in wooden containers. The steaming treatment was carried on for 1 hour at 15 pounds pressure. The inoculum consisted of a 3-day-old growth of the bacteria on potato-mannitol agar. The maximum survival period found in these experiments approximated 6 months in soil stored outdoors and 4 months in soil stored in the greenhouse, and in each instance the length of survival was about 1 month longer in steamed than in unsteamed soil. The relatively short period of survival of the cane gall bacteria in soil would seem to point to some other mode of overwintering, such as in the plant itself, ut the solution of this problem remains for the future.

THE CAUSAL ORGANISM

The methods given in Pure Culture Study of Bacteria as of 1933–36 were followed except as noted (*15*, *16*). In the studies on physiology, the chief reliance was placed on results given by Pinckard (*13*), whose work on this organism was generally repeated and confirmed.

GROWTH IN CULTURE

When isolations were made from young 3-week-old galls, minute white colonies were visible on the surface of potato-mannitol, potato-dextrose, or yeast-extract-dextrose agar after 3 to 5 days, and after 2 to 3 days when isolations were made from the organism in culture. At the end of 10 days at 24° C. the colonies were circular, smooth, entire, and raised, with the largest not much more than 4 mm. in diameter when the number of colonies to the plate approximated 100. Submerged colonies were disk-shaped (double convex) and approached half the size of surface colonies.

When aniline-blue agar was employed, it was not uncommon for many of the colonies to take up the dye, but this did not prove to be of positive diagnostic value.[6] The aniline-blue medium did serve to distinguish the cane gall from the crown gall and hairy root organisms. When streaks were made on the surface of this agar, the cane gall organism, like the crown gall organism, took up the dye, but the hairy root organism did not. Although both absorbed the dye, the cane gall organism made much less growth than the crown gall organism and produced a narrow, thin, almost flat streak in contrast to the broad raised streaks produced by the other two organisms.

In nutrient-broth cultures of the cane gall organism a uniform moderate turbidity was produced in about 2 days, but no pellicle was formed. On potato-mannitol-agar slants a moderate, filiform, glistening white growth developed along the streak in 48 hours. The edges were entire and the consistency of the growth was at first watery to butyrous. Upon aging the growth became a dull creamy white, spread irregularly, flattened, and changed to a tough leather consistency.

The cane gall organism grows slowly as compared with other plant pathogens, an observation also made by Pinckard (*13*). In a stndy of growth rates (*9*), it was demonstrated that this organism grew more

[6] Because both crown gall and cane gall bacteria take up aniline-blue dye readily when streaked on aniline-blue agar, it was thought that these bacteria might possibly take up the dye when isolations were attempted with poured plates. However, after repeated experiment, it was found that when dilution plates were poured, from none to many colonies took up the dye and the organisms from dyed as well as from undyed colonies were pathogenic. Hendrickson et al. (7) also noted that crown gall bacteria have strains which do not absorb dye. This phase of the work deserves further study.

slowly than any of 11 species of plant pathogens, having a minimum generation time of 155 minutes.

MORPHOLOGY

When grown in culture media, the cane-gall organism is a rod with rounded ends usually appearing singly or in pairs but sometimes occurring as short chains. Banfield (*4*), however, observed that when present in host tissue these bacteria are always grouped in chains or in masses and do not occur singly. After having grown on potato-mannitol-agar slants for 48 hours at 24° C. the cells were found to have a mean size of 1.72 μ by 0.64 μ when mounted with congo red negative stain. A repetition of the measurements of 6 of the 13 cultures taken at random from the collection gave a mean size of 1.72 μ by 0.63 μ. For each culture, 100 individual cells were measured.

The organism was motile in several liquid and solid media. Presumptive evidence for motility was obtained when turbidity was observed at considerable distances from the center in tubes of soft potato-mannitol agar (0.3 percent agar) which had been seeded by stab puncture. Following the isolation of single cells in potato-mannitol agar and nutrient broth, the appearance and progress of motility of the progeny cultures was more pronounced in broth than on agar, and on the fourth, fifth, and sixth days after isolation into microculture than earlier.

Polar flagella were demonstrated by means of Gray's and Casares-Gil's flagella stains. The former proved better but still not entirely satisfactory because of difficulty in getting the flagella to take up the dye. Several long flagella were commonly observed near one of the poles in a subpolar position. Only rather rarely were they observed at both poles and in these cases it is possible that the cells were nearing the fission point. Some of the flagella were 10 times as long as the individual cells.

PHYSIOLOGY

The work of Pinckard (*13*) on the utilization of various sources of carbon and nitrogen [7] was repeated and verified by the use of his mineral salt yeast-extract basal medium. Attempts to use the Society of American Bacteriologists' basal medium or to substitute simpler substances, such as *d*-glutamic acid, asparagine, inositol, ascorbic acid, and thiamin for yeast extract, were unsuccessful. All tests were made in quadruplicate. Acid was produced from arabinose, xylose, rhamnose, fructose, mannose, galactose, glucose, lactose, and erythritol. An alkaline reaction appeared with melezitose, starch, inulin, pectin, lactositol, calcium gluconate, formic acid, acetic acid, propionic acid, glycollic acid, malonic acid, succinic acid, tartaric acid, malic acid, and yeast extract only. Cellulose was not fermented.

The cane gall organism appears to be able to use most readily the more complex nitrogen compounds such as ferric ammonium citrate, uric acid, oxamide, succinimide, *l*-asparagune, *l*-tyrosine, *l*-cystine, *d*-glutamic acid, and yeast extract.

Gelatin was not liquefied. On litmus milk a slight serum zone, pink color, acid and curd were produced. Nitrates were not utilized

[7] The writer retested all of Pinckard's carbon sources except aesculin, phloridzin, and calcium gluconate but did not make any determinations on titratable acidity.

and nitrites not produced in the synthetic nitrate medium listed in the Manual of Methods of the Society of American Bacteriologists (*14*). When grown in Bacto-nutrient broth there was a slight test for ammonia (Hansen's method *16, p. 12*). In Bacto-tryptone broth negative tests were obtained for hydrogen sulphide production with ZoBell's method (*15, p. 11*) and for indole with Gnezda's technique (*15, p. 10*). Starch was not hydrolyzed and casein not digested. In agar shake cultures, only slight turbidity developed below the surface, placing the organism somewhere between an aerobe and a facultative anaerobe as to oxygen requirements. No apparent gas was produced. The organism when grown in nutrient broth had a thermal death point of about 56° C.

TAXONOMY

The cane gall organism is considered to be a member of the genus *Phytomonas* and the name *Phytomonas rubi* n. sp. is proposed for it. Synonyms (according to the classifications used by plant pathologists) would be *Bacterium rubi* n. sp. and *Pseudomonas rubi* n. sp.

Technical Description

Phytomonas rubi n. sp.

Small, Gram-negative, non-acid-fast rod (1.72 μ by 0.64 μ) with rounded ends, chiefly occurring singly and in pairs, occasionally in short chains in culture and in chains and masses but not singly in host tissue. Weak facultative anaerobe with optimum growth at 27° C.; thermal death point about 56° C.; motile by subpolar flagella; spores not formed.

On potato-mannitol-agar slants growth slow, moderate, filiform, white to creamy-white, with butyrous consistency later becoming leathery. Uniform clouding of bouillon cultures in 36 to 48 hours. Gelatin not liquefied; starch not hydrolyzed; nitrates not reduced but slight ammonia produced in nutrient broth; hydrogen sulfide and indole not formed. Acid produced in milk. Acid but no apparent gas from arabinose, xylose, rhamnose, fructose, mannose, galactose, glucose, lactose, erythritol. Alkali but no apparent gas from melezitose, starch, inulin, pectin, lactositol, calcium gluconate, formic acid, acetic acid, propionic acid, glycollic acid, malonic acid, succinic acid, tartaric acid, malic acid, and yeast extract.

Although it also adsorbs dye, it is readily distinguished from crown gall organism when streaked on aniline-blue agar; slow-growing with minimum generation time of about 155 minutes.

Pathogenic on black and purple raspberries, blackberries, and, to much lesser extent, on red raspberry.

DISCUSSION

Cane gall has some things in common with crown gall, especially in gross symptomatalogy and in certain phases of the life history of the causal organisms. *Phytomonas rubi* is distinctly different from *P. tumefaciens* in its physiology and pathogenicity, including growth character on artificial media, a preference for complex nitrogen sources in its metabolism, and a restricted host range. These and other characteristics summarized in table 1 constitute a basis for giving it specific rank. The characteristic of motility by subpolar flagella may have significance in determining its taxonomic position, but for the present it is placed in the genus *Phytomonas*.

At present, cane gall is of minor importance in New York, thanks largely to the efficiency of the inspection service and extension work. Control measures that are adequate for crown gall are easily adequate for cane gall. One reason may be that cane gall generally occurs upon

the visible parts of the canes, which facilitates eradication; another that the cane gall organism apparently is less able than the crown gall organism to survive in soil. Obviously this latter observation needs further study. Prof. L. M. Cooley, formerly at the Geneva (N. Y.) Experiment Station, who cooperated with the writer in the field in the course of these observations on raspberry diseases, noted a progressive decline in cane gall in western New York plantings, and at the present time his successor, Dr. R. F. Suit, has infrequently encountered it in commercial plantings in areas where it was formerly present.

TABLE 1.—*Differential characteristics of the cane gall organism (Phytomonas rubi n. sp.), and the crown gall organism (P. tumefaciens)*

Differential characters	Cane gall organism	Crown gall organism
Symptoms on naturally infected plants.	Galls found at and above soil level	Galls found at and below soil level.
	Galls discolor and decompose rapidly; ordinarily unable to isolate organism after July 1.	Galls, while subject to decomposition, may persist throughout season.
	Long gall ridges or galls distributed indiscriminately on canes.	Galls generally occur individually.
	Bacteria migrate up through outer tissue of cane.	Bacteria unable to migrate up the cane in same manner.
Symptoms on artificially infected plants.	Very small galls on roots	Large galls on roots.
	Small galls below soil level on canes.	Large galls at and below soil level on canes.
	Large galls above ground	Small galls above ground.
Longevity of organism in soil	Few months	1 to 3 years.
Host range	Limited genus *Rubus*	Very wide; includes many plant families.
Minimal incubation period	2½ to 3 weeks	10 days to 2 weeks.
Motility of bacteria	Motile	Doubtful motility in many cases.
Stability of bacteria in culture.	Unstable; pathogenicity frequently lost within a year.	Very stable; pathogencity retained for 10 years and longer.
Minimal generation time of bacteria.	155 minutes	78 minutes.
Growth in:		
Nutrient-dextrose broth	Weak growth, slight or no pellicle.	Strong growth, heavy pellicle.
Potato-mannitol agar	Thin leathery growth with time	Abundant growth, watery to butyrous.
Nitrogen metabolism	Unable to use potassium nitrate, ammonium nitrate, ammonium sulfate, potassium nitrite, urea, dicyandiamide and acetamide.	Ammonium nitrate, ammonium sulfate, potassium nitrite, urea, dicyandiamide, and acetamide support excellent growth.
Reaction on litmus milk	Slight serum zone	Heavy serum zone.
	Pink color	Grayish brown.
	Acid	Neutral.

SUMMARY

Cane gall, a rather widely distributed but economically relatively unimportant disease of *Rubus* spp., has been investigated and a description given of its symptoms. The characteristic beading and elongate gall ridges on the above-ground canes are in marked contrast to crown gall which ordinarily occurs at or below ground level. The causal organism was readily isolated from young galls, its pathogenicity was proved, and it was studied in detail. The name proposed for the pathogen is *Phytomonas rubi* n. sp. The organism was found to be pathogenic on black raspberry, purple raspberry, blackberry, and red raspberry, but only weakly so on the last-named species upon which it rarely occurs in the field.

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EFFECT OF ENVIRONMENTAL FACTORS ON THE TRANSPIRATION AND GROWTH OF TOMATO PLANTS[1]

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INTRODUCTION

It is generally held that the transpiration rate of plants is largely determined by the environmental factors that surround the plant, but there is still much uncertainty concerning the very large loss of water through transpiration.

The factors that affect the rate of movement of water from the soil into the plant, the upward movement within the plant, and the ultimate dissipation as vapor into the surrounding air are all fully discussed in a voluminous literature that is readily available. The effects of environment on transpiration have been studied by Alexandroff (*1*),[2] Briggs and Shantz (*2, 3, 4*), Burgerstein (*5, 6*), Clapp (*7*), Curtis (*8*), Dixon (*9*), Iljin (*11*), Kiesselbach (*12*), Lloyd (*13*), Maximov (*14*), Miller (*15*), Snow (*18*), Sresnevski (*19*), Udolskaja (*20*), and others. However, there appear to be few data recorded in the literature concerning the effect on the transpiration rate of the exhaustion of soil nutrients and the consequent retardation of the growth of the plant. Furthermore, it is apparent that considerable misleading data have been used for interpreting the facts relating to this problem. Data have appeared which indicate that plants grown in soils low in nitrogen, as compared with those grown in soils high in nitrogen, consistently have the higher transpiration ratio (quantity of water transpired per gram of aerial dry matter formed) and also the higher transpiration rate (amount of water lost in absolute units per unit of leaf area per unit of time); and these data have been used as a basis for illogical interpretation of the facts. This paper presents the results of studies that show why the interpretation is incorrect.

METHODS AND MATERIALS

CULTURAL CONDITIONS

All data to be presented were collected from experiments previously reported by the writers (*10*). The experiments were conducted in the greenhouses at the Arlington Experiment Farm, Arlington, Va., during 1934. The tomato plants were grown in a good greenhouse compost soil consisting of loam from Arlington farm, manure, and muck, with which sand was later mixed to improve water penetration and distribution. The water-retaining capacity of this soil remained uniform at 65 percent of its dry weight throughout the study.

The plants were grown at soil-moisture levels of approximately 38, 47, and 56 percent of the weight of the soil, representing 59, 72, and 86 percent of the moisture-holding capacity, respectively. The rate of

[1] Received for publication August 5, 1940.
[2] Italic numbers in parentheses refer to Literature Cited, p. 719.

water loss for each plant was recorded daily. At the same time efforts were made to maintain the above-mentioned soil-moisture levels by frequent (4 to 6 times daily) replacement of water lost as indicated by weighing the cultures on solution balances of 20- to 40-kg. capacity. The soil surface of each culture was covered with a close-fitting disk of waterproof paper to reduce evaporation losses.

The plants were grown in glazed 3-gallon crocks, without drainage, holding 12 kg. of soil. The fertilizers were mixed with the soil at the rate of 2 tons per acre as it was placed in the crocks. The fertilizer formulas used were 12–0–12, 12–6–12, and 6–12–6, the figures in each representing percent of nitrogen, phosphoric acid, and potash, respectively. A series of check cultures received no fertilizer.

In each of the three temperature units of the greenhouse (66°, 70°, and 74° F.) and each of the three moisture levels at each temperature the following cultures were set up: Five crocks of each of two fertilizer treatments, six crocks of the third treatment, and three unfertilized checks. Thus, at each of the 36 points of observation there were 3 to 6 replicate cultures, giving a total of 171 plants under moderately well controlled environments.

The plants (Marglobe variety) were pruned to a single stem, tied to bamboo stakes and topped just above the sixth flower cluster.

GROWTH HABIT AND RESPONSE OF TOMATO PLANTS TO ENVIRONMENT

The plants used in these experiments were similar to those grown under field conditions. They were mature and large, with heavy thick foliage, and they bore a good crop of fruit. In the high-temperature unit (74° F.) the plants were tall and slender with relatively small stems and long internodes; they were succulent and of the spindling type. In the medium-temperature unit (70°) the plants were shorter, the stems were heavier, and the leaves were spaced closer together. In the lowest temperature unit (66°) the plants were still shorter, had dense green foliage, and many showed the strongly vegetative type of growth.

TEMPERATURE AND HUMIDITY

It was intended to operate the greenhouse units at 75°, 70°, and 65° F., respectively, from the beginning of the experiment, September 10, but high outside temperature made it impossible to keep the units at these temperatures until about the first week in October. For the duration of the experiment the average weekly mean temperatures were 74.3°, 70.4°, and 65.7° in units 1, 2, and 3, respectively. From October 2 to December 17, during which period better temperature control could be maintained, the averages of the weekly means were 74.6°, 69.1°, and 62.9°, respectively.

The averages of weekly mean relative humidities of units 1, 2, and 3 for the entire period were 55.3, 60.0, and 63.1 percent, respectively. This order of difference unavoidably existed throughout the period regardless of the seasonal temperature, since there was no available means of raising the moisture content of the air introduced from outside incidental to ventilation and temperature control.

ATMOMETER INDEX

In table 1 the atmometer readings for the different temperature units are given. There appear to be considerably larger differences

between the black and white atmometers in the higher temperature units than in the lower ones. These differences can be explained if the concept of Sresnevski (*19*) is accepted; he reported that such differences must be proportional to the deficit of saturation.

TABLE 1.—*Corrected daily average atmometer readings on a weekly basis from three temperature units, 1934*

Period ended	Daily average atmometer evaporation from—							
	Unit 1 (74° F.) [1]				Unit 2 (70° F.)		Unit 3 (66° F.)	
	Black	White	Black	White	Black	White	Black	White
	Ml.	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
Sept. 24	21.2	14.4	20.6	14.5	19.5	15.1	18.8	14.
Oct. 1	21.1	14.6	21.0	15.1	19.6	15.4	19.4	16.
Oct. 8	18.0	12.6	18.4	13.1	16.1	12.9	15.9	12.
Oct. 15	25.5	18.3	26.2	19.0	22.9	18.0	22.4	18.
Oct. 22	20.8	15.5	22.0	15.9	18.8	15.3	18.2	14.
Oct. 29	19.7	15.6	20.4	15.4	16.2	14.0	14.9	12.
Nov. 5	21.0	15.5	20.1	14.6	17.5	14.4	15.5	12.
Nov. 12	20.5	16.3	21.5	15.7	15.4	13.3	13.1	11.
Nov. 19	19.7	16.0	20.3	16.1	16.5	14.3	14.4	12.
Nov. 26	15.2	11.9	16.3	12.5	12.7	11.3	11.1	10.
Dec. 3	11.7	9.2	12.0	9.3	7.8	7.4	6.3	5.
Dec. 10	18.8	14.7	18.7	14.3	14.8	13.7	12.3	11.
Dec. 17	22.9	18.8	22.2	18.5	17.9	17.0	14.7	14.
Average	19.7	14.8	19.9	14.9	16.5	14.0	15.1	12.

[1] Two sets of atmometers installed.

STATISTICAL ANALYSIS OF DATA

The data were analyzed statistically by the analysis of variance method as adapted by Snedecor (*16, 17*), and all significant differences were based on the generalized error. Differences required for significance between means of treatments and single treatments were calculated when the error appeared to be homogeneous. Significant differences are indicated by odds of 19:1 at the 5-percent level of *t*; highly significant differences are indicated by odds of 99:1 at the 1-percent level of *t*. Also, in tables 2 to 7, inclusive, the third and fourth decimals have been rounded off in the customary way.

EXPERIMENTAL RESULTS

To gain a better conception of the responses of the tomato plant grown under different environmental conditions, a large number of data were collected bearing upon water requirement, total water transpired, final dry weight of aerial parts, fruit yield, and starch content of stem and leaf parts of the plants from the different cultures, all of which are essential for an adequate interpretation of the data on daily amounts of water transpired, presented later in this paper.

EFFECT OF ENVIRONMENT ON WATER REQUIREMENT OF THE TOMATO

The term "water requirement" of plants has been used by many investigators to express the ratio between water absorbed (or water expenditure) and dry matter produced. However, in the data presented in table 2 this term is used in the same sense in which it was employed by Briggs and Shantz (*2, 3, 4*), to indicate the milliliters of water transpired per gram of gain in dry weight of aerial vegetative parts of plants in the several environments. It is well known that many factors may influence the water requirement of plants, which varies widely with different species.

TABLE 2.—*Effect of soil moisture, soil nutrients, and temperature on the water requirement of tomato plants, Sept. 18 to Dec. 28, 1934*

[Data based on weighted averages of 3 to 6 plants in each culture]

EXPERIMENTAL DATA

Soil moisture [1] (percent)	Fertilizer formula (N-P-K)	Water requirement [2] at—			
		74° F.	70° F.	66° F.	Mean
		Ml.	*Ml.*	*Ml.*	*Ml.*
59	12-0-12	579	450	317	448.7
	12-6-12	559	398	302	419.7
	6-12-6	572	491	446	503.0
	Check	668	632	535	611.7
	Mean	594.5	492.8	400.0	495.8
72	12-0-12	629	493	409	510.3
	12-6-12	620	472	366	486.0
	6-12-6	678	535	452	555.9
	Check	721	648	551	640.0
	Mean	662.0	537.0	444.5	547.8
86	12-0-12	694	558	445	565.7
	12-6-12	685	547	438	556.7
	6-12-6	721	618	486	608.3
	Check	786	721	590	699.0
	Mean	721.5	611.0	489.8	607.4
Mean	12-0-12	634.0	500.3	390.3	508.2
	12-6-12	621.3	472.3	368.7	487.4
	6-12-6	657.0	548.0	461.3	555.4
	Check	725.0	667.0	558.7	650.2
	Mean	659.3	546.9	444.8	550.3

DIFFERENCES REQUIRED FOR SIGNIFICANCE

Item	Odds	
	19:1	99:1
	Ml.	*Ml.*
Between single cultures	58.8	82.4
Between means of 3 cultures	34.0	47.6
Between means of 4 cultures	29.4	41.2
Between means of 9 cultures	19.6	27.5
Between means of 12 cultures	17.0	23.8

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square
Total	35	14,624
Between moisture	2	[3] 37,465
Between temperature	2	[3] 138,243
Between fertilizer	3	[3] 47,197
Total interactions	28	673
Moisture × temperature	4	510
Moisture × fertilizer	6	390
Temperature × fertilizer	6	[4] 1,681
Remainder (error)	12	364

[1] Percentage of water-retaining capacity.
[2] Mililiters of water per gram of dry weight of tops.
[3] Significant with odds greater than 99:1.
[4] Significant with odds greater than 19:1 but less than 99:1.

The data given in table 2 show the highly significant effects of soil moisture, temperature, and soil nutrients on the water requirement of

he tomato plants in the respective cultures. The mean value of 550.2 for the check plants under all conditions was significantly different from the 6–12–6 mean of 555.4, the 12–6–12 mean of 487.4, and he 12–0–12 mean of 508.2. (See table 2 for differences required for significance at odds of 19:1 and 99:1.) A statistical interpretation of hese data indicates quite definitely that the unfertilized group of plants, consistently and uniformly, had the highest water requirement or the entire growth period under all environments studied. Furthermore, these data appear to indicate that the rate of transpiration of he unfertilized check plants averaged higher than that of any of the ertilized groups of plants during the entire growth period.

As is shown by the data in table 2, variations in environmental conitions, such as are determined by fertilizer, soil moisture, humidity, nd air temperature, may cause a wide variation in the expenditure f water by plants. Low soil moisture and low air temperature have reat influence in reducing the water consumption per gram of dry aterial produced; furthermore, increasing amounts of nitrogen along ith potash promote greater efficiency in water utilization. Increasng the soil moisture results in more extravagant use of water by the lants. It is significant that in these studies plants without added ertilizer were found to be the most extravagant users of water under ll cultural conditions. This has long been a misinterpreted phenomnon. It has misled investigators into assuming that there was no hange in the relative rate of transpiration of the check plants when oil nutrients were exhausted, or at any stage of their growth. It is lso important to note that the 12–0–12 fertilizer cultures expended lightly larger amounts of water than did the 12–6–12 cultures. This act will be illustrated in more detail in figures 2 to 11, to be presented ater.

EFFECT OF ENVIRONMENT ON TOTAL WATER TRANSPIRED

The data given in table 3 show the marked influence of environment n the total amount of water transpired by the tomato plants in the ifferent cultures during the 96-day period, September 18 to December 3, 1934. The difference required for significance between means of ine plant cultures at odds of 99:1 was 3.06 liters. The total water ranspired by the check plant cultures with mean of 53.30 liters of ater was highly significant, and greater than the 6–12–6 mean of 8.18 liters, the 12–6–12 mean of 39.71 liters, and the 12–0–12 mean f 41.44 liters. Further study of the analysis of variance shows a ighly significant interaction of moisture × temperature and temperture × fertilizer, as measured from the generalized error. The magitude of differences between individual plant cultures is also apparent.

EFFECT OF ENVIRONMENT ON DRY WEIGHT

Table 4 gives data showing the effect of environment on the total ry weight of tomato plants grown in the different cultures. Since hese plants were topped after the appearance of the sixth flower luster and since all axillary growth was removed promptly as it ppeared, the final dry weights of the plants in the different cultures ere in fairly close agreement except where indicated by the data in able 4. It is important to note that there were no significant differnces in dry weight between the means of the check plants at different

temperatures except the 66.46 mean at 59 percent soil moisture, but that there were interactions between moisture × fertilizer with odds greater than 99:1.

TABLE 3.—*Effect of soil moisture, soil nutrients, and temperature on the total water transpired by tomato plants, Sept. 18 to Dec. 28, 1934*

[Data based on weighted averages of 3 to 6 plants in each culture]

EXPERIMENTAL DATA

Soil moisture (percent) [1]	Fertilizer formula (N-P-K)	Water transpired at— 74° F.	70° F.	66° F.	Mean
		Liters	*Liters*	*Liters*	*Liters*
59	12-0-12	24.1	23.3	21.9	23.07
	12-6-12	25.3	20.8	19.6	21.91
	6-12-6	31.3	27.6	28.8	29.20
	Check	43.3	39.6	38.5	40.45
	Mean	30.99	27.80	27.17	28.65
72	12-0-12	43.4	39.4	35.7	39.49
	12-6-12	41.9	38.5	38.8	39.74
	6-12-6	54.5	49.2	46.5	50.03
	Check	65.5	54.8	49.3	56.49
	Mean	51.31	45.45	42.55	46.44
86	12-0-12	71.9	60.4	53.1	61.78
	12-6-12	66.3	54.1	52.1	57.49
	6-12-6	72.3	61.0	62.7	65.33
	Check	71.5	58.8	58.6	62.97
	Mean	70.48	58.57	56.64	61.89
Mean	12-0-12	46.45	40.99	36.89	41.44
	12-6-12	44.52	37.81	36.81	39.71
	6-12-6	52.66	45.90	46.00	48.18
	Check	60.08	51.06	48.78	53.30
	Mean	50.93	43.94	42.12	45.66

DIFFERENCES REQUIRED FOR SIGNIFICANCE

Item	Odds 19:1	99:1
	Liters	*Liters*
Between single cultures	6.55	9.17
Between means of 3 cultures	3.77	5.30
Between means of 4 cultures	3.27	4.58
Between means of 9 cultures	2.18	3.06
Between means of 12 cultures	1.89	2.65

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square
Total	35	249.4928
Between moisture	2	[2] 3,319.9205
Between temperature	2	[2] 259.4527
Between fertilizer	3	[2] 353.7813
Total interactions	28	18.2914
Moisture × temperature	4	[2] 30.8991
Moisture × fertilizer	6	[2] 50.9000
Temperature × fertilizer	6	4.8218
Remainder (error)	12	4.5193

TABLE 4.—*Effect of soil moisture, soil nutrients, and temperature on the total dr weight of tomato plants, 1934*

EXPERIMENTAL DATA

[Data based on weighted averages of 3 to 6 plants in each culture]

Soil moisture (percent)[1]	Fertilizer formula (N-P-K)	Dry weight of plants at—			
		74° F.	70° F.	66° F.	Mean
		Gm.	*Gm.*	*Gm.*	*Gm.*
59	12-0-12	41.6	51.7	68.9	54.
	12-6-12	45.4	52.3	64.9	54.
	6-12-6	54.6	56.1	64.6	58.
	Check	64.8	62.7	71.9	66.
	Mean	51.60	55.69	67.56	58.
72	12-0-12	69.0	79.8	87.3	78.
	12-6-12	67.7	81.7	105.8	85.
	6-12-6	80.3	91.9	102.8	91.
	Check	90.8	84.8	89.4	88.
	Mean	76.97	84.55	96.34	85.
86	12-0-12	103.6	108.2	119.4	110.
	12-6-12	96.7	98.9	118.8	104.
	6-12-6	100.2	98.7	129.2	109.
	Check	90.9	81.5	99.4	90.
	Mean	97.87	96.84	116.71	103.
Mean	12-0-12	71.42	79.90	91.88	81.
	12-6-12	69.92	77.63	96.50	81.
	6-12-6	78.39	82.22	98.85	86.
	Check	82.19	76.34	86.91	81.
	Mean	75.48	79.02	93.54	82.

DIFFERENCES REQUIRED FOR SIGNIFICANCE

Item	Odds	
	19:1	99:1
	Gm.	*Gm.*
Between single cultures	15.3	21.
Between means of 3 cultures	8.82	12.
Between means of 4 cultures	7.64	10.
Between means of 9 cultures	5.10	7.
Between means of 12 cultures	4.41	6.

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square
Total	35	485.68
Between moisture	2	[2] 6,313.16
Between temperature	2	[2] 1,098.21
Between fertilizer	3	58.85
Total interactions	28	71.42
Moisture × temperature	4	29.12
Moisture × fertilizer	6	[2] 191.68
Temperature × fertilizer	6	73.01
Remainder (error)	12	24.59

[1] Percentage of water-retaining capacity.
[2] Significant with odds greater than 99:1.

EFFECT OF ENVIRONMENT ON FRUIT YIELD

Yield of fruit was affected somewhat markedly by the different er vironments, as is indicated by the data given in table 5. The frui yield from the check plants was significantly smaller than that fror any of the fertilized group of plants, except at 59 percent soil moisture at odds greater than 99 : 1. The analysis of variance indicates th highly significant effect of soil moisture, temperature, and fertilizer o the yield of fruit from the plants in the different cultures; also, tl

interaction of soil moisture × fertilizer was highly significant. Optimum conditions for fruit yield appeared to be 70° F. and 86 percent soil moisture. The magnitude of difference in yield of fruit due to treatment is large enough to be apparent even between individual treatments. These data are given to indicate the amounts of water required by developing fruit.

TABLE 5.—*Effect of soil moisture, soil nutrients, and temperature on the average yield of green fruit per tomato plant, 1934*

EXPERIMENTAL DATA

Soil moisture (percent)[1]	Fertilizer formula (N-P-K)	Weight of fruit per plant at— 74° F.	70° F.	66° F.	Mean
		Gm.	*Gm.*	*Gm.*	*Gm.*
59	12-0-12	658	826	533	672.3
	12-6-12	603	825	576	668.0
	6-12-6	1,072	1,088	1,087	1,082.3
	Check	1,092	1,078	1,023	1,064.3
	Mean	856.2	954.2	804.7	871 7
72	12-0-12	1,511	1,621	1,324	1,485.3
	12-6-12	1,500	1,587	1,380	1,489.0
	6-12-6	1,935	2,026	1,677	1,879.3
	Check	1,378	1,360	1,250	1,329.3
	Mean	1,581.0	1,648.5	1,407.5	1,545.7
86	12-0-12	2,238	2,238	1,820	2,098.6
	12-6-12	2,269	2,293	1,947	2,169.6
	6-12-6	2,382	2,284	2,220	2,295.3
	Check	1,520	1,528	1,393	1,480.3
	Mean	2,102.2	2,085.7	1,845.0	2,011.0
Mean	12-0-12	1,469.0	1,561.6	1,225.6	1,418.7
	12-6-12	1,457.3	1,568.3	1,301.0	1,442.2
	6-12-6	1,796.3	1,799.3	1,661.3	1,752.3
	Check	1,330.0	1,322.0	1,222.0	1,291.3
	Mean	1,513.1	1,562.8	1,352.5	1,476.1

DIFFERENCES REQUIRED FOR SIGNIFICANCE

Item	Odds 19:1	99:1
	Gm.	*Gm.*
Between single cultures	194.7	273.0
Between means of 3 cultures	112.39	157.64
Between means of 4 cultures	97.37	136.52
Between means of 9 cultures	64.92	91.01
Between means of 12 cultures	56.22	78.82

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square
Total	35	299,563
Between moisture	2	[2] 3,937,240
Between temperature	2	[2] 145,042
Between fertilizer	3	[2] 344,631
Total interactions	28	45,937
Moisture × temperature	4	11,329
Moisture × fertilizer	6	[2] 189,191
Temperature × fertilizer	6	9,641
Remainder (error)	12	3,904

[1] Percentage of water-retaining capacity.
[2] Significant with odds greater than 99:1.

TABLE 6.—*Effect of soil moisture, soil nutrients, and temperature on the starch content of tomato plant stems, 1934*

EXPERIMENTAL DATA

Soil moisture (percent) [1]	Fertilizer formula (N-P-K)	Starch content of stems (on dry-weight basis) at—			
		74° F.	70° F.	60° F.	Mean
		Percent	*Percent*	*Percent*	*Percent*
50	12-0-12	1.54	2.90	8.49	4.31
	12-6-12	1.68	1.95	5.61	3.06
	6-12-6	1.59	2.42	5.56	3.19
	Check	1.27	11.55	22.87	11.90
	Mean	1.52	4.71	10.63	5.62
72	12-0-12	.61	1.67	3.11	1.80
	12-6-12	1.22	.92	2.39	1.51
	6-12-6	1.58	1.65	3.64	2.29
	Check	9.41	12.72	24.14	15.42
	Mean	3.21	4.24	8.32	5.20
86	12-0-12	.46	.58	1.09	.71
	12-6-12	1.33	2.19	2.33	1.95
	6-12-6	1.90	1.82	6.31	3.34
	Check	3.87	6.14	19.54	9.85
	Mean	1.89	2.68	7.32	3.96
Mean	12-0-12	.87	1.72	4.23	2.27
	12-6-12	1.41	1.69	3.44	2.18
	6-12-6	1.69	1.96	5.17	2.94
	Check	4.86	10.14	22.18	12.39
	Mean	2.21	3.88	8.76	4.95

DIFFERENCES REQUIRED FOR SIGNIFICANCE

Item	Odds	
	19:1	99:1
	Percent	*Percent*
Between means of 9 cultures	1.36	1.91
Between means of 12 cultures	1.18	1.66

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square
Total	35	37.29
Between moisture	2	[2] 9.09
Between temperature	2	[3] 139.08
Between fertilizer	3	[3] 222.70
Total interactions	28	12.17
First-degree interactions	16	19.97
Remainder (error)	12	1.76

[1] Percentages of water-retaining capacity.
[2] Significant with odds greater than 19 : 1 but less than 99 : 1.
[3] Significant with odds greater than 99 : 1.

EFFECT OF ENVIRONMENT ON STARCH CONTENT OF STEM AND LEAVES

Large accumulation or storage of starch in the stem and leaf parts of tomato plants is usually an indication of nitrogen deficiency, of retarded metabolism and plant growth, and of the onset of a dehydration process within the plant (*10*). Table 6 gives data showing the effect of environment on the starch content of tomato plant stems.

will be observed that significant differences between means of ·tilizer treatments, of moisture treatments, and of temperatures are ·licated by the high variances due to those single factors as compared th the variances due to second-degree interaction. Since this inter- tion (fertilizer × moisture × temperature) is heterogeneous, it is ·t an adequate basis for determining the magnitude of differences quired for significance except for means of primary effects. Even these instances, the limitations of the estimated magnitudes must kept in mind and unjustified conclusions avoided. The same iitations apply with respect to the data presented in table 7, giving e starch content of the top leaves of the plant.

BLE 7.—*Effect of soil moisture, soil nutrients, and temperature on the starch content of top leaves of tomato plants, 1934*

Soil moisture (percent)[1]	Fertilizer formula (N-P-K)	Starch content of top leaves (on dry-weight basis) at—			
		74° F.	70° F.	66° F.	Mean
		Percent	*Percent*	*Percent*	*Percent*
	12-0-12	3.68	1.17	3.74	2.86
	12-6-12	4.12	1.14	4.72	3.33
	6-12-6	5.16	1.42	3.29	3.29
	Check	3.12	7.98	9.22	6.77
	Mean	4.02	2.93	5.24	4.06
	12-0-12	3.79	1.33	4.84	3.32
	12-6-12	3.52	1.97	4.85	3.45
	6-12-6	5.83	2.13	7.80	5.25
	Check	13.71	11.69	17.15	14.18
	Mean	6.71	4.28	8.66	6.55
	12-0-12	3.02	3.57	4.70	3.76
	12-6-12	2.99	2.53	5.16	3.56
	6-12-6	8.39	6.02	9.57	7.99
	Check	13.45	10.56	15.53	13.18
	Mean	6.96	5.67	8.74	7.12
Mean	12-0-12	3.50	2.02	4.43	3.32
	12-6-12	3.54	1.88	4.91	3.44
	6-12-6	6.46	3.19	6.89	5.51
	Check	10.09	10.08	13.97	11.38
	Mean	5.90	4.29	7.55	5.91

DIFFERENCES REQUIRED FOR SIGNIFICANCE

Item	Odds	
	19:1	99:1
	Percent	*Percent*
een means of 9 cultures	1.46	2.05
een means of 12 cultures	1.26	1.78

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square
	35	17.83
een moixture	2	[2] 31.77
een temperature	2	[2] 31.79
een fertilizer	3	[2] 128.63
interactions	28	3.06
degree interactions	16	5.41
inder (error)	12	2.03

To be observed in table 7 are the highly significant effects of so moisture, fertilizer, and temperature, as indicated by the analysis variance of the data. Furthermore, the mean of 11.38 percent starch for the check culture was significantly higher than the mean 5.51 percent for the 6–12–6 culture, the mean of 3.44 percent for th 12–6–12 culture, and the mean of 3.32 percent for the 12–0–12 cu ture, with odds greater than 99:1. The difference required fc significance between means of nine plant cultures with odds great than 99:1 was 2.05 percent of starch.

EFFECT OF CERTAIN SOIL NUTRIENTS ON THE TRANSPIRATION OF TOMATO PLAN

The data given in table 8 show a rather marked association betwee exhaustion of soil nutrients and retardation of transpiration in th unfertilized plants. Apparently this was caused by changes in th metabolic processes within the plant associated with a rather gradu dehydration of the plant tissue, which is indicated by a large accumula tion of starch (*10*). These data were collected from tomato-plan cultures grown in nine different environments, with 36 points of obse vation, as has been described. They are based on average absolut amounts of water transpired daily during 2- to 4-day periods by thre unfertilized check plants and three plants receiving each specifie fertilizer treatment during the last 64 days of growth, September 1 to November 21, 1934. In table 8 the absolute amounts of wat transpired by the respective cultures—check, 12–0–12, 12–6–12, an 6–12–6—are given in the first four columns. The total water tra spired by each group of cultures is given at the bottom of the respecti tables. The ratios of the transpiration of the several fertilized cu tures to that of the unfertilized check culture for the same period a given in the last three columns of table 8. Since a simple ratio of th absolute units of water transpired by the fertilized plants to th absolute units of water transpired by the unfertilized check plan over similar periods of time is being dealt with, the relative chang in these ratios from day to day indicate the rate of transpiration the plants in the different cultures.

TABLE 8.—*Comparison of the daily transpiration of unfertilized and fertiliz tomato plants grown in nine different soil environments, 1934*

[Data based on total transpiration of 3 plants in each culture at 2- to 4-day periods of the last 65 day growth]

UNIT 1, AT 74° F.; 59 PERCENT SOIL MOISTURE

Period ended—	Water transpired from—				Transpiration ratio of—[1]		
	Unfertilized check	Plants receiving fertilizer—			Plants receiving fertilizer—		
		12–0–12	12–6–12	6–12–6	12–0–12	12–6–12	6–12–
	Ml.	*Ml.*	*Ml.*	*Ml.*			
Sept. 21	2,930	1,800	2,080	2,200	0.614	0.710	0.
Sept. 25	1,600	930	820	950	.581	.513	.
Sept. 27	1,760	820	960	950	.466	.545	.
Sept. 29	1,740	1,090	1,130	1,600	.626	.649	.
Oct. 2	3,100	1,500	1,600	1,950	.475	.506	.
Oct. 5	3,665	2,050	1,925	2,375	.559	.525	.
Oct. 8	3,730	1,625	1,735	2,325	.436	.465	.
Oct. 12	6,600	3,140	3,070	4,150	.476	.465	.
Oct. 15	8,350	4,020	3,940	5,260	.481	.472	.
Oct. 17	4,845	2,275	2,215	2,980	.470	.457	.

TABLE 8.—*Comparison of the daily transpiration of unfertilized and fertil tomato plants grown in nine different soil environments, 1934*—Continued

Period ended	Water transpired from—				Transpiration ratio of—		
	Unfertilized check	Plants receiving fertilizer—			Plants receiving fertilizer		
		12-0-12	12-6-12	6-12-6	12-0-12	12-6-12	6-1
UNIT 1, AT 74° F.; 59 PERCENT SOIL MOISTURE—Continued							
	Ml.	*Ml.*	*Ml.*	*Ml.*			
Oct. 20	6,800	3,225	3,150	4,350	0.474	0.463	
Oct. 22	4,650	2,325	2,325	3,225	.500	.500	
Oct. 24	5,950	3,000	2,850	4,000	.504	.479	
Oct. 26	3,450	1,775	1,775	2,475	.514	.514	
Oct. 29	5,800	3,075	3,025	4,225	.530	.522	
Oct. 31	4,500	2,725	2,675	3,475	.606	.594	
Nov. 2	6,225	4,000	4,000	5,100	.643	.643	
Nov. 4	4,850	3,175	3,100	3,825	.655	.639	
Nov. 6	3,500	2,400	2,250	2,900	.686	.643	
Nov. 8	5,250	3,700	3,350	4,350	.705	.638	
Nov. 10	5,000	3,500	3,250	3,900	.700	.650	
Nov. 12	4,050	2,950	2,750	3,550	.728	.679	
Nov. 14	5,600	4,000	3,650	4,700	.714	.652	
Nov. 16	3,900	3,200	2,550	3,300	.821	.654	
Nov. 19	8,250	6,250	5,500	7,000	.758	.667	
Nov. 21	5,800	4,150	3,600	4,950	.716	.621	
Total	121,955	72,700	69,275	90,065			
UNIT 1, AT 74° F.; 72 PERCENT SOIL MOISTURE							
Sept. 21	3,100	2,120	2,120	2,080	0.684	0.684	
Sept. 25	2,950	1,250	1,030	1,730	.424	.349	
Sept. 27	3,300	1,290	1,090	1,670	.391	.330	
Sept. 29	2,800	1,450	1,000	1,440	.518	.357	
Oct. 2	5,550	2,300	1,990	2,850	.414	.359	
Oct. 5	6,625	2,650	2,075	3,625	.400	.313	
Oct. 8	7,400	2,450	2,090	3,985	.331	.282	
Oct. 12	12,550	4,900	4,060	7,350	.390	.334	
Oct. 15	16,225	6,650	5,650	10,300	.410	.348	
Oct. 17	8,275	3,975	3,400	5,950	.480	.411	
Oct. 20	11,400	5,750	4,900	8,825	.504	.430	
Oct. 22	7,900	4,350	3,525	6,525	.551	.446	
Oct. 24	10,400	5,750	4,600	8,350	.553	.442	
Oct. 26	5,425	3,375	2,950	5,200	.622	.544	
Oct. 29	9,525	6,000	5,125	8,575	.630	.538	
Oct. 31	7,225	5,000	4,400	6,825	.692	.609	
Nov. 2	9,625	7,850	7,350	9,850	.816	.764	1
Nov. 4	7,425	6,300	6,075	7,625	.848	.818	1
Nov. 6	5,100	4,500	4,450	5,400	.882	.873	1
Nov. 8	7,150	6,700	6,750	7,900	.937	.944	1
Nov. 10	7,100	6,100	6,300	7,100	.859	.887	1
Nov. 12	5,250	4,850	4,760	5,450	.924	.905	1
Nov. 14	7,106	6,700	6,850	7,650	.944	.965	1
Nov. 16	5,150	4,550	4,800	5,200	.883	.932	1
Nov. 19	9,950	10,250	10,550	11,050	1.030	1.060	1
Nov. 21	6,500	7,450	7,800	7,900	1.146	1.200	1
Total	191,000	124,510	115,680	160,405			
UNIT 1, AT 74° F.; 86 PERCENT SOIL MOISTURE							
Sept. 21	4,100	2,730	2,840	3,110	0.666	0.693	0
Sept. 25	3,830	1,920	2,090	2,480	.501	.546	.
Sept. 27	4,290	2,060	2,220	2,830	.480	.517	.
Sept. 29	3,590	1,420	1,780	2,350	.396	.496	.
Oct. 2	6,970	3,120	3,790	4,750	.448	.544	.
Oct. 5	7,750	3,800	4,525	5,650	.490	.584	.
Oct. 8	7,925	4,400	4,925	6,150	.555	.621	.
Oct. 12	13,400	7,800	8,800	11,000	.582	.657	.
Oct. 15	15,975	10,950	12,125	14,350	.685	.759	.
Oct. 17	7,795	6,400	6,780	7,660	.821	.870	.
Oct. 20	11,550	10,000	10,450	11,275	.866	.905	.
Oct. 22	7,950	7,375	7,775	8,325	.928	.978	1.
Oct. 24	11,400	10,550	10,700	11,500	.925	.939	1.
Oct. 26	5,775	5,650	5,750	6,225	.978	.996	1.
Oct. 29	10,975	10,350	10,400	11,300	.943	.948	1.
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE 8.—*Comparison of the daily transpiration of unfertilized and fertilized tomato plants grown in nine different soil environments, 1934*—Continued

UNIT 1, AT 74° F.; 86 PERCENT SOIL MOISTURE—Continued

Period ended	Water transpired from—				Transpiration ratio of—		
	Unfertilized check	Plants receiving fertilizer—			Plants receiving fertilizer—		
		12-0-12	12-6-12	6-12-6	12-0-12	12-6-12	6-12-6
	Ml.	*Ml.*	*Ml.*	*Ml.*			
Nov. 2	11,300	12,475	12,075	13,250	1.104	1.069	1.173
Nov. 4	8,425	9,950	9,700	10,325	1.181	1.151	1.226
Nov. 6	5,600	7,350	6,850	7,100	1.312	1.223	1.268
Nov. 8	8,850	10,750	9,750	9,950	1.215	1.102	1.124
Nov. 10	7,800	9,800	8,650	8,800	1.256	1.109	1.128
Nov. 12	6,250	7,850	7,100	7,550	1.256	1.136	1.208
Nov. 14	8,150	10,500	9,150	10,000	1.288	1.123	1.220
Nov. 16	5,150	8,100	6,450	6,900	1.573	1.252	1.340
Nov. 19	9,650	16,150	13,750	13,850	1.674	1.425	1.435
Nov. 21	6,800	11,500	9,800	9,750	1.691	1.441	1.434
Total	209,675	201,450	196,575	215,530			
UNIT 2, AT 70° F.; 59 PERCENT SOIL MOISTURE							
Sept. 21	3,200	1,700	1,900	2,100	0.531	0.594	0.656
Sept. 25	1,910	510	620	780	.267	.325	.408
Sept. 27	2,340	1,090	1,170	1,260	.466	.500	.538
Sept. 29	1,630	750	830	950	.460	.509	.583
Oct. 2	4,040	1,540	1,630	1,950	.381	.403	.483
Oct. 5	4,315	1,475	1,545	1,940	.342	.358	.450
Oct. 8	3,780	1,370	1,450	1,760	.362	.384	.466
Oct. 12	7,590	2,325	2,380	3,265	.306	.314	.430
Oct. 15	8,300	2,950	2,820	3,825	.355	.340	.461
Oct. 19	7,700	2,800	2,850	3,800	.364	.370	.494
Oct. 21	5,075	1,875	1,950	2,550	.369	.384	.502
Oct. 23	4,525	1,650	1,925	2,400	.365	.425	.530
Oct. 25	5,250	1,975	2,250	2,900	.376	.429	.552
Oct. 29	6,375	2,625	2,875	3,775	.412	.451	.602
Nov. 1	6,375	3,200	3,425	4,200	.502	.537	.659
Nov. 3	4,950	2,900	2,950	3,650	.586	.596	.737
Nov. 5	4,300	2,675	2,625	3,275	.622	.610	.762
Nov. 7	4,650	3,050	2,900	3,700	.656	.624	.796
Nov. 9	4,150	2,500	2,350	3,000	.602	.566	.723
Nov. 12	6,050	3,675	3,475	4,675	.607	.574	.773
Nov. 14	4,100	2,550	2,350	3,250	.622	.573	.793
Nov. 16	3,050	2,150	1,900	2,450	.705	.623	.803
Nov. 19	7,400	5,600	4,600	6,400	.757	.622	.865
Nov. 22	5,300	3,800	3,300	4,450	.717	.623	.840
Total	116,355	56,735	56,070	72,305			
UNIT 2, AT 70° F., 72 PERCENT SOIL MOISTURE							
Sept. 21	2,790	2,070	1,840	2,170	0.742	0.659	0.778
Sept. 25	2,510	1,240	1,180	1,740	.494	.470	.693
Sept. 27	3,040	1,450	1,180	1,890	.477	.388	.622
Sept. 29	2,100	1,040	830	1,200	.475	.397	.548
Oct. 2	4,860	2,370	1,780	2,910	.488	.366	.599
Oct. 5	5,450	2,430	2,105	3,460	.456	.386	.635
Oct. 8	5,600	2,180	1,910	3,160	.389	.341	.564
Oct. 12	11,225	4,825	4,475	6,975	.430	.399	.621
Oct. 15	12,725	5,750	5,175	8,300	.452	.407	.652
Oct. 19	11,300	5,700	5,250	8,400	.504	.465	.743
Oct. 21	7,575	4,300	4,050	5,700	.568	.535	.752
Oct. 23	6,625	4,125	3,975	5,500	.623	.600	.830
Oct. 25	7,825	5,025	4,550	6,625	.642	.581	.847
Oct. 29	9,025	6,550	5,875	8,050	.726	.651	.892
Nov. 1	9,675	7,575	6,725	9,250	.783	.695	.956
Nov. 3	7,700	6,300	6,050	8,050	.818	.786	1.045
Nov. 5	6,725	5,800	5,725	7,125	.862	.851	1.059
Nov. 7	6,950	6,550	6,450	7,700	.942	.928	1.108
Nov. 9	5,350	5,700	5,650	6,300	1.065	1.056	1.178
Nov. 12	7,550	7,875	7,750	8,200	1.043	1.026	1.086
Nov. 14	4,800	5,350	5,250	5,450	1.115	1.094	1.135
Nov. 16	4,050	4,050	4,125	4,425	1.000	1.019	1.093
Nov. 19	8,850	10,400	10,650	11,150	1.175	1.203	1.260
Nov. 22	6,825	7,200	7,400	7,225	1.055	1.084	1.059
Total	161,215	115,855	109,950	140,955			

TABLE 8.—*Comparison of the daily transpiration of unfertilized and fertilized tomato plants grown in nine different soil environments, 1934*—Continued

UNIT 2, AT 70° F.; 86 PERCENT SOIL MOISTURE

Period ended	Water transpired from—				Transpiration ratio of—		
	Unfertilized check	Plants receiving fertilizer—			Plants receiving fertilizer—		
		12-0-12	12-6-12	6-12-6	12-0-12	12-6-12	6-12-6
	Ml.	*Ml.*	*Ml.*	*Ml.*			
Sept. 21	4,030	2,260	2,620	3,330	0.561	0.650	0.826
Sept. 25	3,760	1,810	2,070	2,690	.481	.551	.715
Sept. 27	4,260	2,180	2,350	3,090	.512	.552	.725
Sept. 29	2,860	1,300	1,450	2,150	.455	.507	.752
Oct. 2	6,550	3,260	3,370	4,940	.498	.515	.754
Oct. 5	7,175	3,650	3,800	5,925	.509	.530	.826
Oct. 8	6,200	3,550	3,400	5,350	.573	.548	.863
Oct. 12	13,250	7,975	7,675	11,425	.602	.579	.862
Oct. 15	13,125	9,500	9,000	12,600	.724	.686	.960
Oct. 19	11,500	9,500	9,050	11,550	.826	.787	1.004
Oct. 21	7,025	6,875	6,450	7,850	.979	.918	1.117
Oct. 23	6,825	6,475	5,900	7,125	.949	.864	1.044
Oct. 25	7,875	7,825	7,000	8,425	.994	.889	1.070
Oct. 29	9,800	9,675	8,775	10,475	.987	.895	1.069
Nov. 1	9,550	11,025	9,925	11,275	1.154	1.039	1.181
Nov. 3	7,900	9,350	8,550	9,650	1.184	1.082	1.222
Nov. 5	6,400	8,475	7,675	8,225	1.324	1.199	1.285
Nov. 7	7,000	9,100	8,500	9,050	1.300	1.214	1.293
Nov. 9	6,200	8,050	7,250	7,000	1.298	1.169	1.129
Nov. 12	7,950	10,600	9,700	9,575	1.333	1.220	1.204
Nov. 14	4,900	7,150	6,650	6,400	1.454	1.357	1.306
Nov. 16	3,975	6,050	5,700	5,225	1.522	1.434	1.314
Nov. 19	8,600	15,200	13,850	11,950	1.767	1.610	1.390
Nov. 22	7,000	10,900	9,850	9,200	1.557	1.407	1.314
Total	173,710	171,735	160,560	184,475			

UNIT 3, AT 66° F.; 59 PERCENT SOIL MOISTURE

Sept. 21	3,320	1,920	2,200	2,400	0.578	0.663	0.723
Sept. 25	2,000	500	550	790	.250	.275	.395
Sept. 29	4,430	2,180	1,840	2,745	.492	.415	.620
Oct. 2	4,260	1,730	1,800	2,420	.406	.423	.568
Oct. 5	4,500	1,365	1,590	2,310	.303	.353	.513
Oct. 8	4,240	1,360	1,210	1,920	.321	.285	.453
Oct. 12	8,200	2,610	2,555	4,350	.318	.312	.530
Oct. 15	8,050	2,820	2,660	4,550	.350	.330	.565
Oct. 19	7,550	2,725	2,500	4,475	.361	.331	.593
Oct. 22	7,550	2,750	2,500	4,800	.364	.331	.636
Oct. 24	5,900	2,200	1,950	4,100	.373	.331	.695
Oct. 26	3,550	1,350	1,250	2,350	.380	.352	.662
Oct. 30	7,100	2,950	2,650	5,000	.415	.373	.704
Nov. 1	4,200	2,000	1,750	3,200	.476	.417	.762
Nov. 3	5,550	2,800	2,600	4,200	.505	.468	.757
Nov. 5	4,150	2,450	2,300	3,550	.590	.554	.855
Nov. 7	5,300	3,250	3,000	4,700	.613	.566	.887
Nov. 9	3,300	2,300	2,200	3,275	.697	.667	.992
Nov. 11	4,250	3,075	3,000	4,275	.724	.706	1.006
Nov. 13	2,450	1,950	1,875	2,575	.796	.765	1.051
Nov. 15	2,900	2,600	2,425	3,225	.897	.836	1.112
Nov. 17	4,950	4,400	4,250	5,750	.889	.859	1.162
Nov. 20	4,450	4,250	4,350	5,550	.955	.978	1.247
Nov. 22	1,600	1,950	2,000	2,150	1.219	1.250	1.344
Total	113,750	57,485	55,005	84,660			

UNIT 3, AT 66° F.; 72 PERCENT SOIL MOISTURE

Sept. 21	3,040	2,210	2,250	2,760	0.727	0.740	0.908
Sept. 25	2,520	1,360	1,250	1,730	.540	.496	.687
Sept. 29	5,545	2,900	2,800	3,965	.523	.505	.715
Oct. 2	4,960	2,780	2,325	3,410	.560	.469	.687
Oct. 5	5,450	2,625	2,375	3,700	.482	.436	.679
Oct. 8	5,300	2,380	2,250	3,500	.449	.425	.660
Oct. 12	11,300	5,325	4,850	7,450	.471	.429	.659
Oct. 15	10,800	5,600	5,300	8,000	.519	.491	.741
Oct. 19	9,375	5,625	5,475	7,750	.600	.584	.827
Oct. 22	9,750	6,050	6,000	8,300	.627	.615	.851

TABLE 8.—*Comparison of the daily transpiration of unfertilized and fertilized tomato plants grown in nine different soil environments, 1934*—Continued

UNIT 3, AT 66° F.; 72 PERCENT SOIL MOISTURE

Period ended	Water transpired from—				Transpiration ratio of—		
	Unfertilized check	Plants receiving fertilizer—			Plants receiving fertilizer—		
		12-0-12	12-6-12	6-12-6	12-0-12	12-6-12	6-12-6
	Ml.	*Ml.*	*Ml.*	*Ml.*			
Oct. 24	7,850	5,300	5,150	6,950	0.675	0.656	0.885
Oct. 26	4,475	3,125	3,075	3,975	.698	.687	.888
Oct. 30	9,500	6,700	6,950	8,600	.705	.732	.905
Nov. 1	5,000	4,100	4,200	4,950	.820	.840	.990
Nov. 3	6,750	5,500	5,950	7,000	.815	.881	1.037
Nov. 5	5,000	4,750	4,950	5,600	.950	.990	1.120
Nov. 7	6,400	6,150	6,450	7,400	.961	1.008	1.156
Nov. 9	4,325	4,375	4,700	5,125	1.012	1.087	1.185
Nov. 11	5,325	5,350	5,725	6,156	1.005	1.075	1.155
Nov. 13	3,100	3,175	3,275	3,800	1.024	1.056	1.226
Nov. 15	3,650	3,925	4,075	4,800	1.075	1.116	1.315
Nov. 17	5,800	7,050	7,500	7,800	1.216	1.293	1.345
Nov. 20	5,500	7,150	7,800	7,600	1.300	1.418	1.382
Nov. 22	2,600	3,000	3,200	3,050	1.154	1.231	1.193
Total	143,315	106,505	107,875	133,365			

UNIT 3, AT 66° F.; 86 PERCENT SOIL MOISTURE

Period ended	Unfertilized check	12-0-12	12-6-12	6-12-6	12-0-12	12-6-12	6-12-6
Sept. 21	5,320	3,260	3,200	4,140	0.613	0.602	0.778
Sept. 25	4,020	2,030	1,760	2,700	.505	.438	.672
Sept. 29	9,330	4,560	4,035	6,255	.489	.432	.670
Oct. 2	7,690	3,890	3,140	5,430	.506	.408	.706
Oct. 5	7,750	4,000	3,550	5,925	.516	.458	.765
Oct. 8	7,400	3,900	3,300	5,600	.527	.446	.757
Oct. 12	15,175	8,850	7,225	12,025	.583	.476	.792
Oct. 15	13,300	9,200	7,400	11,800	.692	.556	.887
Oct. 19	11,025	8,850	7,325	10,875	.803	.664	.986
Oct. 22	10,000	9,350	7,550	10,900	.935	.755	1.090
Oct. 24	8,250	8,000	6,450	9,200	.970	.782	1.115
Oct. 26	4,800	4,750	4,075	5,775	.990	.849	1.203
Oct. 30	10,000	10,500	8,900	12,450	1.050	.890	1.245
Nov. 1	5,350	5,900	5,100	6,800	1.103	.953	1.271
Nov. 3	7,450	8,150	7,300	9,450	1.094	.980	1.268
Nov. 5	5,200	6,500	5,750	6,950	1.250	1.106	1.337
Nov. 7	7,100	8,300	7,750	9,550	1.169	1.092	1.345
Nov. 9	4,725	5,850	5,425	6,375	1.238	1.148	1.349
Nov. 11	5,825	7,375	6,850	8,125	1.266	1.176	1.395
Nov. 13	3,275	4,225	3,975	4,825	1.290	1.214	1.473
Nov. 15	3,975	5,275	4,975	5,525	1.327	1.252	1.390
Nov. 17	6,400	9,100	8,900	9,600	1.422	1.391	1.500
Nov. 20	6,200	9,100	8,800	9,300	1.468	1.419	1.500
Nov. 22	2,750	4,050	3,650	3,950	1.473	1.327	1.436
Total	172,310	151,965	136,385	183,525			

It is obvious that the daily transpiration of all plants increased with increase in size, and later declined during the cloudy months of fall and winter; however, it is also apparent that certain of the check plants had a greater decline in their daily transpiration than did the corresponding fertilized plants. There was some difference in the time when the plants were topped, but this difference was due primarily to the effect of soil moisture and temperature and not to fertilizer treatment. The majority of the plants were topped during the last two weeks of October before any considerable decline in transpiration occurred. Topping the plants after the appearance of the sixth flower cluster produced plants of nearly the same size and weight. The relative plant sizes are indicated by the data given in table 4 on the dry weight of the plants.

A detailed interpretation of the data presented in table 8 can best be made by diagrams or graphs. Figure 1 illustrates the gradual change in the rate of transpiration of the check plants, associated apparently with the exhaustion of nutrients in the soil, as compared with the 12–6–12 fertilized plant culture. This diagram is based on

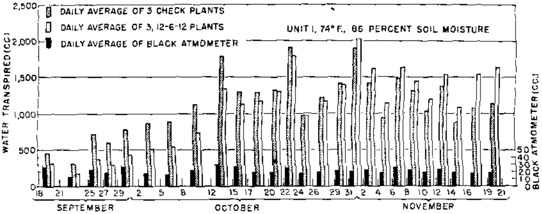

FIGURE 1.—Daily transpiration of three fertilized (12–6–12) and of three unfertilized tomato plants averaged during 2- to 4-day periods for the last 64 days of growth, with corrected black-atmometer index to indicate the relative evaporation intensity during the same periods. Plants grown in greenhouse unit 1, at 74° F. and 86 percent soil moisture.

the average daily amounts of water transpired per plant over 2- to 4-day periods during the 64 days of growth at 74° F. Marked changes in the rate of transpiration were indicated, although the soil-moisture content was maintained at a fairly constant level of approximately 86 percent of water-retaining capacity. The corrected atmometer index is shown for the period in order to give some idea of

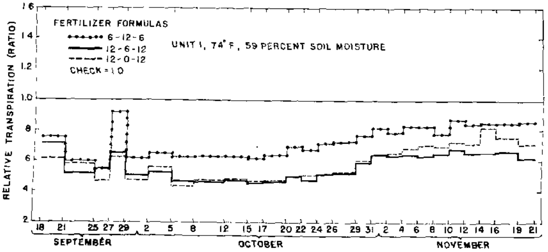

FIGURE 2.—Relative transpiration of tomato plants fertilized with different amounts of nutrients, in comparison with the unfertilized check taken as unity. Based on data from table 8, unit 1, 74° F., 59 percent soil moisture.

the evaporation intensities. The amount of water transpired per day from the check plants exceeded that from the 12–6–12 fertilized plants during the first 43 days. After the forty-third day, October 31, there was a sufficient decrease in the daily transpiration of the check plants to cause it to fall below the level of the 12–6–12 plant culture

while the transpiration of the latter continued with comparatively slight change.

The data given in table 8 relating to the ratios of the transpiration of the fertilized to that of the respective unfertilized check cultures of tomatoes are presented graphically in figures 2 to 10.

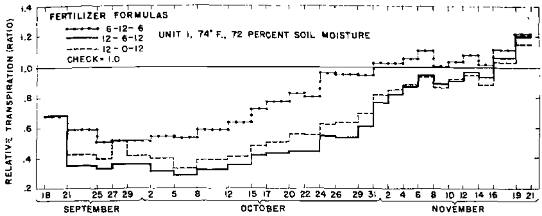

FIGURE 3.—Relative transpiration of tomato plants fertilized with different amounts of nutrients, in comparison with the unfertilized check taken as unity. *Based on data from table 8, unit 1, 74° F., 72 percent soil moisture.*

With the check plants equaling 1.0 as a basis for comparison, figures 2 to 10 show the rather marked changes in the transpiration ratios of the fertilized to the respective unfertilized check plants. It is important to observe that whereas the check plants transpired much more water than did the fertilized plants of about the same

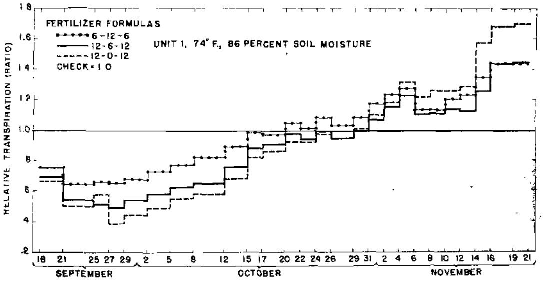

FIGURE 4.—Relative transpiration of tomato plants fertilized with different amounts of nutrients, in comparison with the unfertilized check taken as unity. Based on data from table 8, unit 1, 74° F., 86 percent soil moisture.

size, as indicated by early and total amounts, their transpiration fell off gradually in many cases, while the fertilized plants (still about the same size as the checks) did not show an equal decline in the amount of transpiration; therefore, in such cases the checks gradually dropped below the fertilized plants. These trends are definite and are reflected in the increasing values of the ratios based on the check

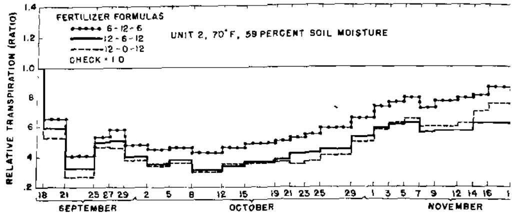

FIGURE 5.—Relative transpiration of tomato plants fertilized with diff amounts of nutrients, in comparison with the unfertilized check taken as u Based on data from table 8, unit 2, 70° F., 59 percent soil moisture.

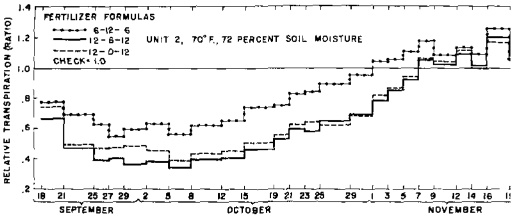

FIGURE 6.—Relative transpiration of tomato plants fertilized with diff amounts of nutrients, in comparison with the unfertilized check taken as u Based on data from table 8, unit 2, 70° F., 72 percent soil moisture.

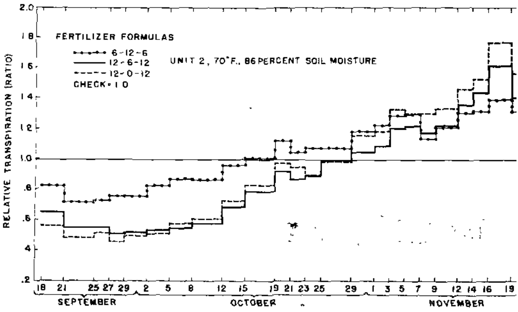

FIGURE 7.—Relative transpiration of tomato plants fertilized with diffe amounts of nutrients, in comparison with the unfertilized check taken as u Based on data from table 8, unit 2, 70° F., 86 percent soil moisture.

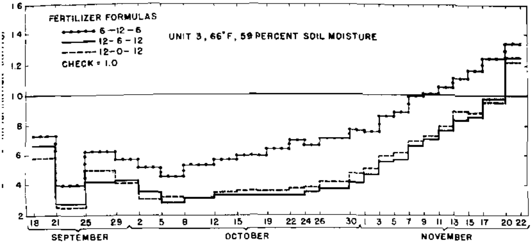

FIGURE 8.—Relative transpiration of tomato plants fertilized with different amounts of nutrients, in comparison with the unfertilized check taken as unity. Based on data from table 8, unit 3, 66° F., 59 percent soil moisture.

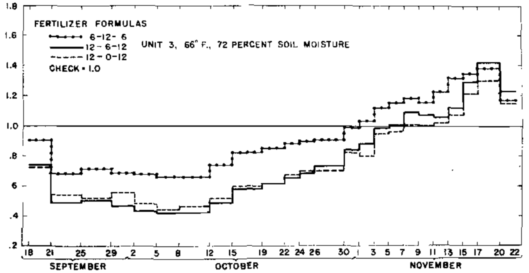

FIGURE 9.—Relative transpiration of tomato plants fertilized with different amounts of nutrients, in comparison with the unfertilized check taken as unity. Based on data from table 8, unit 3, 66° F., 72 percent soil moisture.

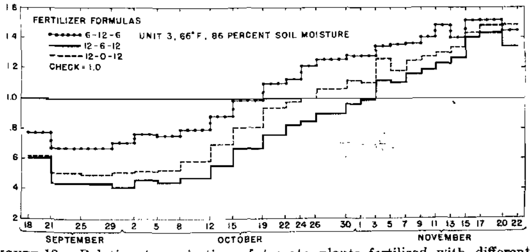

FIGURE 10.—Relative transpiration of tomato plants fertilized with different amounts of nutrients, in comparison with the unfertilized check taken as unity. Based on data from table 8, unit 3, 66° F., 86 percent soil moisture.

plants as unity. Once the ratio lines cross the unity base line they do not recross; and there is little or no subsequent downward trend.

This change in ratio of water transpired was rather marked even with low soil moisture, 59 percent (figs. 2, 5, and 8); but at this moisture level the ratio of transpiration of the fertilized to the check plants exceeded 1.0 only when the plants were grown at 66° F. The marked changes in the transpiration of the check plants are interesting, since the dry weights of the check plants exceeded those of the fertilized ones at all temperatures when grown at a low soil-moisture level (table 4).

The effect of 72 percent soil moisture was to hasten the relative transpiration change, as indicated by figures 3, 6, and 9. At this soil-moisture level there was a more rapid change in the ratios of transpiration and also there were larger differences between the transpiration ratios of the several treatments than was the case at a 59-percent soil-moisture level. There was also an increase in dry weights of the plants in these moister cultures, as is indicated by data in table 4.

The transpiration change occurred at an even earlier date when the plants were grown with 86 percent soil moisture. There was also a more rapid growth of the plants, which would tend to exhaust the soil nutrients earlier (figs. 4, 7, and 10).

It is obvious from the data given that many factors affect growth response and transpiration. Attention should be called to the deleterious effects of low soil moisture (59 percent of water-retaining capacity), at which level growth was retarded rather markedly by the applied fertilizer—evidently toxic under these cultural conditions because of the high osmotic strength of the soil solution. This fact is indicated by the larger dry weight of the check plants and the greater use of water per unit of dry weight. It also appears that growth of the check plants was retarded to such an extent that the soil nutrients were not totally exhausted; consequently, there was a relatively small change in the transpiration of the check plants at this low soil-moisture level. However, at the low temperature (66° F.) the transpiration of the check plants exceeded that of the fertilized plants until after the fifty-third and sixty-fourth day, when the declining transpiration of the check plants dropped below that of the fertilized plants (fig. 8). The close similarity under all conditions of the daily transpiration of the two fertilized cultures 12–0–12 and 12–6–12 is striking, but this phenomenon is difficult to explain adequately (*20*). This relation of the transpiration of these cultures is apparent in figures 2 to 10, inclusive, with the curves for the 12–0–12 cultures closely following those for the 12–6–12 cultures.

The growth rate of the plants at 72 percent soil moisture exceeded that of the plants grown at 59 percent soil moisture at all temperatures, and as a result soil nutrients were exhausted earlier and more rapidly. The faster growth of these plants at 72 percent soil moisture was associated with a more rapid and earlier decline in the transpiration of the check plants when compared with the fertilized plants than was the case at the 59-percent soil-moisture level (figs. 3, 6, and 9). At the high temperature (74° F.), the rate of transpiration of the check plants exceeded that of the 12–0–12 and 12–6–12 fertilized plants until November 19, and that of the 6–12–6 plants until November 2, after

which dates the transpiration of the fertilized cultures exceeded that of the check. At 70° the rate of transpiration of the check plants exceeded that of the 12–0–12 and 12–6–12 fertilized cultures until November 9, and that of the 6–12–6 until November 3, after which dates the fertilized plants transpired larger amounts of water over equal periods. These facts appear to indicate a definite measurable decline in the rate of transpiration of the check plants after nutrient depletion in the soil. The rate of transpiration of the check plants grown at 66° exceeded that of the 12–0–12 plants until November 9, that of the 12–6–12 plants until November 7, and that of the 6–12–6 plants until November 3, after which dates the rate of transpiration of the fertilized plants exceeded that of the check plants.

The largest increase in plant growth and fruit yield occurred with plants grown at 86 percent soil moisture in all three temperature units. At 74° F. the rate of transpiration of the check plants exceeded that of the 12–0–12 fertilized plants until October 31, that of the 12–6–12 plants until November 2, and that of the 6–12–6 plants until October 22 (figs. 4, 7, and 10). At 70° the rate of transpiration of the check plants exceeded that of the 12–0–12 and 12–6–12 fertilized cultures until November 1, and that of the 6–12–6 culture until October 19, after which dates there was a definite measurable decline in the rate of transpiration of the check plants (fig. 7). At 66° the rate of transpiration of the check plants exceeded that of the 12–0–12 fertilized plants until October 30, that of the 12–6–12 plants until November 5, and that of the 6–12–6 plants until October 22 (fig. 10). These facts are a further indication of a definite measurable decline in the transpiration of unfertilized plants that occurred concurrently with the exhaustion of soil nutrients.

DISCUSSION

The extensive data presented in both table and graph emphasize the effects of soil moisture, soil nutrients, and temperature on the general growth habits of the tomato, on the total water expenditure, on the daily transpiration, on periodic changes in the daily amount of transpiration, and on the starch content of the plants. It is obvious that additional data would be helpful in drawing conclusions. Measurements of the leaf area of the plants in the respective cultures would have been valuable but were impossible to obtain with so large a group of plants. The only available data showing comparative size of unfertilized and fertilized plants are those in table 4 showing the dry weights of the vegetative part of the plants. Dry and green weights of the leaf part of the plants are available, but these would not be adequate for determining the rate of transpiration per unit area, since it is assumed that some transpiration takes place through the stem. Furthermore, the dry weight of the leaf part of the plant shows a straight-line relationship to the total vegetative dry weight of the plant, hence the two are equally serviceable as criteria of size. The data given in tables 2 to 4 show striking differences in magnitude because of fertilizer, soil moisture, and temperature differences. One important and notable exception is that no significant differences due to fertilizer are indicated by the mean square of the dry weight of the plants, but interaction involving fertilizer × moisture is highly significant (table 4). However, fertilizer had a highly significant effect

(with odds greater than 99:1) on the water requirement (table 2) and on the total water transpired (table 3) during the experimental period. These data in tables 2 to 4 and the statistical analysis included with each table are very good examples of data that may lead one astray in attempting an explanation of a series of events when only the end result is observed. In tables 2 and 3, in the analysis of variance highly significant differences are indicated which to the casual observer might lead to the general conclusion that the check plants had the highest rate of transpiration per plant throughout the entire period of the experiment. Data presented in table 8 and the graphic presentation of these data in figures 2 to 10 show very strikingly why such an interpretation would be incorrect. It is quite evident that a rather marked decline in the transpiration of the check plants occurred concurrently with the exhaustion of the soil nutrients and nitrogen (*10*).

Data on water requirement for entire growth periods have been used as a basis for the following concept: "When the supply of nutrients in the soil approaches exhaustion, the rate of growth of the plant is greatly reduced, but no corresponding change occurs in the transpiration rate. This fact is evidence that transpiration is not a measure of growth" (*15, p. 500*). Data that give only the final water requirement (or water expenditure per gram of gain in plant weight) are not valid for supporting the concept just quoted. Data based on the total water transpired cannot possibly reveal what happened to the rate of transpiration of the plants before or after the soil nutrients were exhausted or during any other limited period; they indicate merely that the check plants averaged a relatively higher rate of transpiration over the entire test period.

The large accumulation of starch in the stem and leaf parts (tables 6 and 7) of the unfertilized check plants was highly significant and differed from that of the fertilized group of tomato plants with odds greater than 99:1. These data appear to indicate that a definite change in the metabolism of the check plants has resulted because of the apparent exhaustion of soil nutrients. These data also suggest that a process of dehydration was initiated, with a large part of the carbohydrate content of the check plants becoming inactive, a further indication of retarded metabolism (*10*). The decline in the daily transpiration of the check plants was concurrent with this apparent retarded metabolism and growth, and is illustrated by the data presented in table 8 and in figures 2 to 10. Lloyd (*13*) and Iljin (*11*) called attention to the fact that high starch content of the leaves was usually associated with stomatal closing, and these authors suggested a possible relation to retarded transpiration.

SUMMARY

In this paper extensive data are submitted that show differences in the daily amount of transpiration of tomato plants (*Lycopersicon esculentum* Mill.) when grown in the greenhouse in soil with 4 nutrient treatments under 9 sets of environmental conditions involving 3 variations in soil moisture and 3 in air temperature, giving altogether 36 points of observation for study. Evaporation capacities of the air under the three conditions of temperature were measured.

With increasing amounts of soil moisture and decreasing temperature, there was a corresponding increase in the rate of plant growth that was directly correlated with the early exhaustion of soil nutrients in the unfertilized cultures—a condition which was further reflected by a definite, measurable decline in the transpiration of the unfertilized plants. This decline in transpiration was relatively large at high soil-moisture levels.

Data treated statistically by the method of analysis of variance indicate the highly significant effects of soil moisture, soil nutrients, and temperature on total water transpired, on the water requirement, on the fruit yield, and on the starch content of stem and leaf parts of tomato plants. Temperature and soil moisture, but not soil nutrients, had a highly significant effect on the final dry weight of the plants, all of which were topped uniformly after the appearance of the sixth flower cluster and pruned for removal of all axillary growth.

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EFFECT OF CERTAIN FUNGICIDES AND ENVIRONMENTAL FACTORS ON THE RATE OF TRANSPIRATION OF TOMATO PLANTS[1]

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INTRODUCTION

The effect on rate of transpiration of the application of certain fungicides to the foliage of plants has attracted considerable attention during the past 25 years. Recently, however, this interest has become more intense because of the effect of a number of these fungicides, especially those containing zinc sulfate or copper phosphate, on the growth and metabolic responses of the plant. In many of the experiments reported, the plants used were young, the foliage was tender, and the apparent increase in rate of transpiration was due to injury by the fungicide and subsequent desiccation and cuticular loss, as suggested by Horsfall and Harrison (*17*).[2] At the present time it appears that when certain fungicides are applied to young, tender plants there is a definite measurable increase in the rate of transpiration, but when applied to old, mature plants the rate of transpiration changes little. Very few data have appeared in the literature that show the effects of these fungicides on the rate of transpiration of large, bearing plants, when grown under widely different environmental conditions.

Since fungicides are also applied to large, bearing plants when grown under field conditions in commercial practice, the rate of transpiration and the metabolic responses of the plants under these conditions are of considerable importance. Therefore, any additional quantitative data secured under conditions approaching those of normal crop plant growth, where the effects of several environmental factors affecting the rate of transpiration are measured concurrently with the effects of certain fungicides, would appear to be desirable in evaluating and interpreting many factors involved in this problem.

In this paper, data are presented showing the independent effects and interactions of fungicides and environmental factors on the rate of transpiration of maturing tomato plants (*Lycopersicon esculentum* Mill.)

REVIEW OF LITERATURE

The literature relating to the effect of fungicides on the rate of transpiration of plants is extensive and varied. The following workers have all reported an increase in the rate of transpiration due to the application of fungicides either on detached leaves or, in many experiments, on young potted plants: Bain (*1*), Bonde (*3*, *4*), Butler (*5*), Duggar and Cooley (*9*, *10*), Duggar and Bonns (*8*),

[1] Received for publication August 30, 1940.
[2] Italic numbers in parentheses refer to Literature Cited, p. 733.

Dutton and Wells (*11*), Frank and Krüger (*15, 16*), Krausche and Gilbert (*18*), Martin (*21*), Martin and Clark (*22*), Runnels and Wilson (*25*), Shive and Martin (*27*), Wilson and Runnels (*30, 31, 32*), and Zucker (*33*).[3] A number of other investigators have failed to find very marked effects of fungicides on the rate of transpiration when, in most instances, normal or field types of plants were used. Among these are Bayer (*2*),[4] Childers (*6*), Clinton (*7*), Ewert (*12*), Horsfall and Harrison (*17*), Lutman (*19, 20*), Miller (*23*), Rumm (*24*), Schander (*26*), and Sturgis (*29*).

MATERIALS AND METHODS

ENVIRONMENTAL CONDITIONS

The tomato plants used in the three experiments reported herein were grown in a fertile greenhouse soil at the Arlington Experiment Farm, Arlington, Va., during 1933 and 1934. The controlled factors were temperature, soil moisture, and the proportions of mineral nutrients. The fungicidal sprays studied were copper phosphate-bentonite-lime mixture, zinc sulfate-lime, and bordeaux mixture.

The experimental method used has been described in detail in previous papers (*13, 14*). The tomato plants were grown at a number of different soil-moisture levels, which will be indicated later. Each of the experimental series was set up by first determining the original amount of water in the soil and then adding sufficient water to bring the water content up to the required percentage. After the seedlings had been transplanted to the crocks the soil moisture was maintained at approximately the desired level by frequent additions (four to six times daily) of measured amounts of water, the required amounts being determined by weighing the cultures on solution balances of 40-kg. capacity.

The plants used were similar to staked and pruned plants grown under field conditions. They were fruiting, large, and had heavy thick foliage. Figure 1 shows the very marked effect of temperature on the growth and habit of the tomato plants used in these experiments; all other conditions were uniform, with soil moisture at 86 percent of water-retaining capacity and with 12-6-12 fertilizer. Figure 2 shows the effect of soil moisture on plant growth and habit when other conditions were uniform.

Three crops of tomatoes were used in the experiments, and the experimental methods differed considerably in the three tests. For convenience in presenting the data, the three experiments will be designated as the copper phosphate, zinc-lime, and bordeaux mixture experiments, and the details will be given under these headings.

All plants in the three experiments were healthy and the fungicides were applied solely to determine their effect on the rate of transpiration.

COPPER PHOSPHATE EXPERIMENT

The copper phosphate experiment was conducted from March 3 to May 24, 1933, and the duration of the water-expenditure record was 82 days. The prespray period extended from March 3 to April 17, or 45 days, and was for the purpose of determining the relative tran-

[3] Cited by Miller (*23*, p. [illegible]).
[4] Cited by Miller (*23*, p. [illegible]).

spiration ratios of the paired plants before spraying. The spray period lasted from April 17 to May 24, or 37 days, during which time the effect of copper phosphate on the rate of transpiration was observed.

FIGURE 1.—Tomato plants showing influence of different temperatures on growth. Cultures grown at (*A*) 65°, (*B*) 70°, and (*C*) 74° F. All were grown at 8[illegible] percent of soil-water-retaining capacity and with 12–6–12 fertilizer.

This crop of tomatoes was grown in two different temperature unit[illegible] of the greenhouse (units 2 and 3), but because of rising outside temper[illegible]

the prespray period the mean temperature of unit 2 was 65.1° F. and that of unit 3 was 68.5°, a difference of 3.4°. During the spray period the mean temperature of unit 2 was 71.4° and that of unit 3 was 72.9°, a difference of only 1.5°. These data indicate a 6.3° mean-temperature difference between the prespray and the spray period for unit 2, and a 4.4° mean-temperature difference for unit 3.

In this experiment, a check and six fertilizer mixtures of the following formulas were used: 0–0–12, 0–6–6, 0–12–0, 6–0–6, 6–6–0, and

FIGURE 2.—Tomato plants showing influence of soil moisture on growth. Cultures grown at (*A*) 65, (*B*) 56, (*C*) 47, and (*D*) 39 percent of soil-water-retaining capacity. All were grown at 76° F. and with 12–2–2 fertilizer. Plants from a series grown in 1932.

12–0–0, the values in each formula representing nitrogen, phosphorus, and potassium, respectively; the check received no additional fertilizer. Five different soil-moisture levels were established and maintained at approximately 86, 77, 69, 60, and 51 percent of the water-retaining capacity of the soil. (See table 1.)

Single plants were grown in 3-gallon glazed crocks. Two crocks of each fertilizer treatment and of the check (receiving no additional fertilizer) were placed in each of the five soil-moisture series in each of the two different temperature units of the greenhouse. One plant of each pair was sprayed and the other was not, giving a treated (T) and a con-

trol (C) plant at each of the 70 points of observation, a total of 140 plants under approximately controlled environmental conditions.

This crop was sprayed with three applications at 10-day intervals during the 37 days of the spray period. The spray mixture consisted of 2 pounds of copper phosphate, 4 pounds of hydrated lime, and 2 pounds of bentonite, mixed and suspended in 50 gallons of water. Approximately 400 cc. of this mixture was applied to each treated plant at each of the three applications. In spraying, the plants were tipped at an angle to avoid application of the spray to the soil. Since the plants were always in the greenhouse, no spray residue was removed by rainfall.

ZINC-LIME EXPERIMENT

The zinc sulfate-lime experiment was conducted on a fall crop of tomatoes from November 23, 1933, to January 22, 1934, a period of 60 days. In this experiment, no prespray period was established since the cultures were of uniform size, permitting pairing at the beginning, one being sprayed and the other not sprayed.

Three fertilizer mixtures of the following formulas were used: 12–6–12, 12–0–12, and 6–12–6. Three soil-moisture levels were established: 86, 77, and 69 percent of the water-retaining capacity of the soil. The plants were grown under three different temperature conditions, and because of favorable outside temperature, inside temperature control was fairly constant. The mean temperatures for units 1, 2, and 3 were 76°, 71°, and 65° F., respectively.

Four crocks (single-plant cultures) of each fertilizer treatment were placed at each of the three soil-moisture levels in each of the three different temperature units of the greenhouse. Two of each of these were sprayed and two were not sprayed, giving two replications at each of the 54 points of observation and a total of 108 plants under well-controlled environmental conditions.

The tomato plants in this experiment were sprayed with a mixture of 4 pounds of zinc sulfate and 4 pounds of hydrated lime in 50 gallons of water. Three applications of this fungicide were made, each at the rate of 400 cc. per treated plant. The first application was made November 23, the date of the first water-expenditure record, and the succeeding applications 2 and 4 weeks later.

BORDEAUX MIXTURE EXPERIMENT

The bordeaux mixture experiment was also made upon a fall crop, the water-expenditure record beginning September 18 and ending December 1, 1934, extending over 74 days. The prespray period lasted 43 days, September 18 to October 31, 1934. The spray period was 31 days, from October 31 to December 1, 1934.

This experiment was different from the other two, as temperature was the only variable in the environment. The mean temperatures for units 1, 2, and 3, were 74°, 70°, and 66° F., respectively. A soil moisture of 72 percent of water-retaining capacity and a 6–12–6 fertilizer were used for the experiment. Three replications at each of the six points of observation gave for study a total of 18 plants.

This crop of tomatoes was sprayed with a 4–4–50 bordeaux mixture, 400 cc. to each treated plant. Three applications were made at 10-day intervals during the spray period.

TREATMENT OF DATA

The statistical procedure used by Duggar and Cooley (*9, 10*) and adopted by Miller (*23*) and others for measuring the effect of fungicides upon the rate of transpiration of plants was followed in the computation and interpretation of the data presented in this work. This method was designed to remove the influence of all factors except those due to spray, i. e., the ratio between the transpiration from the treated and the control plant (T : C). In addition to the calculation of the transpiration ratios, the results of other statistical treatment of the data are also given, namely, the ratio of units of water transpired to the units of dry material produced above ground (water requirement); the water expended during the prespray, spray, and the entire experimental period. It is important to observe that the Duggar-Cooley and the analysis of variance computations appear to give consistent results; consequently either method is a valid basis for the interpretation of data.

Duggar and Cooley (*9, 10*) divided their plants into two groups, designating those to be sprayed "T" (treated) and the control plants "C." These plants were paired and the transpiration ratio T′: C′ was determined before spraying. After the standardization period, the treated plants, T, were sprayed, and the ratio of the units of transpiration T : C was again determined for each pair of plants. If the ratio T : C had increased after spraying, the transpiration rate increase was presumed to be due to the application of the fungicide. If the ratio T : C had decreased, if the transpiration rate was less, or if it had not changed there was no apparent effect of the spraying on the transpiration rate. The third ratio T : C, which is a relative measure of the effect of spraying for the spray period, is then calculated by considering the ratio before spraying as 1.00.

In many of the experiments reported by other workers the amount of water transpired daily by the plants used was small, and the prespray and spray periods were of very short duration. Data obtained under such conditions are difficult to interpret and are not applicable to larger, older plants. To overcome these difficulties bearing plants were used (figs. 1 and 2) that transpired a large volume of water during daily periods, and the prespray and spray periods were extended over several weeks.

The amounts of water transpired over the entire period of observation by all the plants used in these experiments are of interest. During the prespray period of 45 days in the copper phosphate experiment, the average plant transpired a total of 18.3 liters of water; during the spray period of 37 days, 37.5 liters; and for the two periods, a total of 55.8 liters. These amounts are the means for 140 plants (treated and control groups), and if marked stimulation of transpiration had occurred, as has been frequently reported, the large amounts of water recorded would obviously be adequate to determine the different responses.

The tomato plants used in the other two experiments reported herein likewise transpired large amounts of water.

EFFECT OF FUNGICIDES ON TRANSPIRATION RATE OF TOMATO PLANTS

COPPER PHOSPHATE EXPERIMENT

The effect of copper phosphate on the rate of transpiration of tomato plants is illustrated in table 1, which shows the ratios of treated to control (T : C) plants during the prespray period, the spray period, the difference between periods, and the corrected ratio for the spray period. These data are listed under appropriate subheads for each level of soil moisture and each fertilizer under which the spray test was conducted. The application of Snedecor's (*28*) adaptation of the analysis of variance to the ratios (test to control) showed no significant effect of temperature, fertilizer, or soil moisture. However, the difference between the observed treatment mean (1.064) and a hypothetical control mean (1.000) shows the effect of copper phosphate-bentonite-lime spray to be a highly significant factor in increasing water expenditure by odds of more than 99 : 1 (table 2).

To test further the effects of copper phosphate spray on the rate of transpiration of tomato plants, data on the water transpired during the 37 days of the spray period and on the water requirement (total units of water transpired per unit of dry weight of aerial parts of plants) were analyzed by the variance method (table 2). In table 2 the significant effects of copper phosphate spray (test and control groups) on transpiration, and the highly significant effects of temperature, soil moisture, and fertilizer are clearly evident. The following interactions were significant: Temperature × moisture, temperature × fertilizer, and moisture × fertilizer. It is important also to note that there were no significant first-order interactions involving spray treatment, which indicates that the effect of copper phosphate on the rate of transpiration of tomato plants was not influenced significantly by the environments under these experimental conditions.

For the spray period the mean weight of water transpired by the sprayed plants was 38.4 liters and by the unsprayed, 36.7 liters; the mean dry weights were 77.4 gm. and 80.8 gm., respectively. The mean water requirements for the plants under the various conditions were as follows:

	Cc.
Sprayed	749
Unsprayed	703
Greenhouse unit 2 [1]	702
Greenhouse unit 3 [1]	750
At soil moisture percentage:	
86	826
77	735
69	702
60	710
51	658
With fertilizer mixture:	
No fertilizer	781
0–0–12	766
0–6–6	744
0–12–0	784
6–0–6	692
6–6–0	696
12–0–0	620

[1] For temperature of greenhouse unit, see p. 724.

BLE 1. —*Transpiration ratios of control tomato plants and plants sprayed with copper phosphate when grown under different soil moisture and soil nutrient conditions in two different temperature units*

[For temperature of greenhouse unit, see p. 724.]

GREENHOUSE UNIT 2

Fertilizer formula	Transpiration ratios [1] of plants grown in soil of indicated moisture (percentage of saturation)																			
	86				77				69				60				51			
	T':C' before spray	T:C after spray	Relative increase [2]	Ratio increase [3]	T':C' before spray	T:C after spray	Relative increase [2]	Ratio increase [3]	T':C' before spray	T:C after spray	Relative increase [2]	Ratio increase [3]	T':C' before spray	T:C after spray	Relative increase [2]	Ratio increase [3]	T':C' before spray	T:C after spray	Relative increase [2]	Ratio increase [3]
ck	0.899	0.922	1.026	0.023	1.022	1.235	1.208	0.213	0.959	1.008	1.051	0.049	1.026	0.940	0.916	−0.086	0.901	1.033	1.147	0.132
12	.939	1.143	1.217	.204	[4] .998	[4] 1.016	[4] 1.018	[4] .018	.984	1.031	1.048	.047	1.040	1.026	.987	−.014	1.027	.948	.923	−.079
3	1.044	1.013	.970	−.031	.951	1.067	1.122	.116	1.014	1.192	1.176	.178	.973	1.033	1.062	.060	1.105	1.062	.961	−.043
-0	.972	.963	.991	−.009	1.052	1.063	1.010	.011	1.031	1.114	1.081	.083	.918	.986	1.074	.068	1.017	1.081	1.063	.064
3	.781	.954	1.222	.173	[4] .974	[4] .992	[4] 1.018	[4] .018	.982	.981	.999	−.001	.761	1.129	1.484	.368	1.006	1.181	1.174	.175
0	1.118	1.134	1.014	.016	1.029	1.097	1.066	.068	.991	1.131	1.141	.140	1.060	1.071	1.010	.011	1.020	1.146	1.124	.126
-0	.907	.985	1.086	.078	.934	.977	1.046	.043	.924	1.000	1.082	.076	[4] 1.010	[4] 1.082	[4] 1.071	[4] .072	.966	.930	.963	−.036
GREENHOUSE UNIT 3																				
ck	0.938	0.887	0.946	−0.051	1.019	0.756	0.742	−0.263	1.081	1.270	1.175	0.189	1.103	1.108	1.005	0.005	1.062	1.053	0.992	−0.009
12	1.178	1.100	.934	−.078	.891	1.019	1.144	.128	1.086	1.079	.994	−.007	.996	1.031	1.035	.035	.930	1.092	1.174	.162
3	1.006	.870	.865	−.136	1.004	1.058	1.054	.054	1.043	1.014	.972	−.029	.974	1.079	1.108	.105	.871	1.051	1.207	.180
-0	1.191	1.347	1.131	.156	1.091	1.103	1.011	.012	.980	1.018	1.039	.038	1.001	1.105	1.104	.104	[4] .980	[4] 1.082	[4] 1.104	[4] .102
3	.874	1.065	1.219	.191	.969	1.120	1.156	.151	.973	1.016	1.044	.043	.981	1.163	1.186	.182	1.058	1.043	.986	−.015
0	.865	.988	1.142	.123	1.091	1.086	.905	−.005	1.014	1.036	1.022	.022	1.005	1.041	1.036	.036	.894	1.091	1.220	.197
-0	1.150	1.133	.985	−.017	.966	.915	.947	−.051	.990	1.038	1.048	.048	1.015	.993	.978	−.022	.900	1.139	1.266	.239

[1] A difference of 0.340 between 2 ratios is considered significant by odds of 99 : 1.
[2] T : C :: T′ : C′ equals relative transpiration rate.
[3] Absolute increased transpiration ratios due to spray; minus signs indicate decrease.
[4] Control plants sprayed once by error.

TABLE 2.—*Analysis of variance of data showing the effect of copper phosphate spray on the transpiration ratio (T : C and T′ : C′, table 1), total water transpired during spray period, water requirement, and dry weight of tomato plants*

Source of variance	Degrees of freedom	Mean square for—			
		Transpiration ratios	Total water transpired	Water requirement	Dry weight of plants
Total	139	0.008,304	136.52	13,737	552.29
Between spray treatments	1	[1] .123,018	[2] 108.98	[1] 74,199	[2] 389.44
Between temperatures	1	.009,578	[1] 1,145.37	[1] 76,332	[1] 773.62
Between moistures	4	.001,896	[1] 591.20	[1] 108,129	[1] 1,490.43
Between fertilizers	6	.009,459	[1] 2,027.77	[1] 72,035	[1] 8,430.13
Total interactions	127	.007,459	25.13	7,041	150.09
Spray treatments × temperatures	1	.001,872	.01	4,144	107.54
Spray treatments × moistures	4	.002,500	1.29	2,861	9.30
Spray treatments × fertilizers	6	.006,084	5.74	1,238	42.44
Temperatures × moistures	4	.008,154	[1] 23.63	11,910	25.40
Temperatures × fertilizers	6	.014,362	[2] 15.18	[2] 15,915	[2] 179.98
Moistures × fertilizers	24	.008,588	[1] 106.31	8,795	[1] 486.38
Remainder interactions (error)	82	.007,000	5.04	6,797	70.83

[1] Significant by odds of more than 99 : 1.
[2] Significant by odds of more than 19 : 1 but less than 99 : 1.

The application of the copper phosphate spray to tomato plants also had a highly significant effect on the water requirement of the plants as is indicated in table 2. It is of particular interest to note that the effects of copper phosphate, temperature, and fertilizer upon water requirement were of similar magnitude, all being highly significant. Soil moisture also had a highly significant effect. The only significant interaction observed was temperature × fertilizer. Since there was no significant interaction involving spray, it appears that in these studies the effect of spray on water requirement was entirely independent of these environmental factors.

Table 2 also gives a variance analysis of data showing the effects of copper phosphate spray and the environmental factors on the dry weight of the tomato plants. There were significant differences in the dry weights of the sprayed and of the control groups, and highly significant differences due to temperature, moisture, and fertilizer. Rather large interactions were observed for temperatures × fertilizers, and moistures × fertilizers, but there were no significant interactions involving spray treatments.

ZINC-LIME EXPERIMENT

The zinc sulfate-lime experiment differed from the other two experiments reported in this paper. There was no prespray period. The 108 plants were paired at the beginning of the experiment; 54 were sprayed with zinc-lime mixture and the remainder were used as controls. Furthermore, the plants in temperature units 1 to 3 (76°, 71°, and 65° F.) were harvested at different dates (22 days between the 76° and 65° F. lots). These differences in cutting dates interfere with close comparisons of temperature effects as measured by dry weights and total water expended divided by dry weights. However, the data permit the determination of the major effects of the several factors on transpiration during the 60-day period from November 23, 1933, to January 22, 1934.

The mean for the total water transpired was 33.5 liters for the sprayed plants and 33.9 liters for the unsprayed. The analysis of variance of the data on total water transpired during this period

(table 3) indicates that the application of zinc-lime spray had nc effect. However, the differences due to temperature were very large the effects of soil moisture and fertilizer were highly significant and there were highly significant interactions for temperature × soi moisture, temperature × fertilizer, and soil moisture × fertilizer There were no significant interactions involving spray treatment.

TABLE 3.—*Analysis of variance of data, based on the total water transpired, showin the effects of zinc-lime spray and environmental factors on the transpiration o 54 spray and 54 control plants during the period Nov. 23, 1933, to Jan. 22, 193 (60 days)*

Source of variance	Degrees of freedom	Mean square for total water transpired
Total	107	164.8
Between spray treatments (treated and control)	1	4.2
Between temperatures	2	[1] 7,541.9
Between moistures	2	[1] 725.0
Between fertilizers	2	[1] 52.0
Temperatures × moistures	4	[1] 109.4
Temperatures × fertilizers	4	[1] 20.1
Moistures × fertilizers	4	[1] 25.5
Spray treatments × temperatures	2	2.1
Spray treatments × moistures	2	8.2
Spray treatments × fertilizers	2	2.2
Remainder interactions (error)	82	4.3

[1] Significant by odds of more than 99 : 1.

To show further the effects of zinc-lime spray mixture on the rat of transpiration of tomato plants, analysis of variance is given i table 4 for data on "water requirement" (ratio of total units (cubi centimeters) of water transpired to total units (grams) gain in dr weight of aerial part of plant). This table contains data relating t the high-temperature (76° F.) greenhouse unit only. The result showing the effects of spray in the other two units were in agreemen with those in unit 1. Data from all three temperature units were no combined in a single analysis of variance because of the differences i stage of development accompanying different harvest dates. Th mean water requirement for the sprayed plants was 581 cc. per gran of increase in dry weight, and for the control 612 cc., an insignifican difference. The effect of soil moisture on water requirement also wa not significant in this experiment, but fertilizer had a very marke effect which was highly significant. If zinc-lime spray had influence the rate of transpiration (see table 4), this effect probably would hav been reflected in the water requirement data.

TABLE 4.—*Analysis of variance of data on water requirement showing the effects o zinc-lime mixture in the high-temperature unit (76° F.)*

Source of variance	Degrees of freedom	Mean square
Total	35	4,66
Between spray treatments (test and control)	1	8,31
Between moistures	2	6,79
Between fertilizers	2	[1] 32,86
Total interactions	30	2,52
Spray treatments × moistures	2	3,59
Spray treatments × fertilizers	2	1,61
Moistures × fertilizers	4	3,46
Remainder interactions	22	2,33

BORDEAUX MIXTURE EXPERIMENT

The results of the bordeaux mixture experiment are presented in table 5. They show the effects of the treatment on the transpiration ratio of tomato plants under the experimental conditions described. A variance analysis of T : C before and after spraying is given in table 6. The mean T : C for the first (prespray) period was 1.029; for the second (spray) period 1.010; and the mean for the ratio between the two periods, 0.982. A hypothetical mean of 1.000 would indicate no change; although the value 0.982 might suggest a depression of the transpiration rate following spraying, the difference is not significant (table 6).

Additional data show further that bordeaux mixture does not affect the rate of transpiration of mature tomato plants appreciably. The mean water transpired per plant during the spray period by the treated plants was 27.4 liters and by the control plants 27.3 liters. The mean dry weight of the treated plants was 82.4 gm. and that of the controls 82.1 gm., the total water expended was 50.0 and 49.4 liters, respectively, and the mean water requirement 614 cc. and 610 cc., respectively.

TABLE 5.—*Effect of bordeaux mixture on the transpiration ratio of 9 pairs of sprayed and control plants, Sept. 18 to Dec. 1, 1934 (74 days)*

Temperature (°F.)	Transpiration ratio			
	T′: C′ before spray	T: C after spray	Relative increase [1]	Ratio increase [2]
74	1.043	1.002	0.961	−0.041
	1.022	.961	.940	−.061
	.945	.982	1.039	.037
70	.954	1.061	1.112	.107
	.963	.958	.995	−.005
	1.086	.917	.844	−.169
66	1.104	1.127	.968	−.037
	1.008	1.025	1.017	.017
	1.079	1.059	.981	−.020

[1] T : C :: T′: C′ equals relative transpiration rate.
[2] Absolute increased transpiration ratios due to spray; minus signs indicate decrease.

TABLE 6.—*Analysis of variance of data showing the effect of bordeaux spray on total water transpired, dry weight, water requirement, and transpiration ratio (T: C and T′: C′, table 5) of plants*

Source of variance	Degrees of freedom	Mean square for—			
		Total water transpired	Dry weight of plants	Water requirement	Transpiration ratio
Total	17	37.78	68.92	12,140	0.004521
Between spray treatments	1	1.65	.35	61	.001643
Between temperatures	2	[1] 296.63	[1] 221.55	[1] 87,627	[2] .014746
Spray treatments × temperatures	2	6.84	24.69	3,697	.000038
Remainder (error)	12	2.80	56.56	1,973	.003803

[1] Significant by odds of more than 99 : 1.
[2] Significant by odds of more than 19 : 1.

In table 6 data are given showing the lack of significance of the effect of bordeaux mixture on the total water transpired, dry weight of the plants, and water requirement. However, temperature differences

had a highly significant effect on total water transpired and the water requirement, and a significant effect on the dry weight of the plants. It is of particular interest to note that there were no significant interactions of spray × temperature.

Table 7 gives an analysis of variance of the data for the water transpired during the spray period and also during the prespray period. In each test period there was no significant difference in the quantity of water transpired between the two groups of plants (spray and control). Temperature, however, had a highly significant effect. These data on rate of transpiration further indicate that bordeaux mixture had no effect regardless of the method used in attempting to measure effect.

TABLE 7.—*Analysis of variance of data for total water transpired for the prespray and spray periods, showing the effect of bordeaux spray and temperature*

Source of variance	Degrees of freedom	Mean square for water transpired—	
		Prespray period	Spray period
Total	17	4.81	17.28
Between spray treatments	1	1.16	.04
Between temperatures	2	[1] 32.09	[1] 135.73
Spray treatments × temperatures	2	1.22	2.28
Remainder (error)	12	1.17	1.48

[1] Significant by odds of more than 99: 1.

DISCUSSION

The tomato plants used in all three experiments reported in this paper were healthy and free from all traces of parasitic or virus diseases. The three fungicides used were applied solely to determine their effects on rate of transpiration of the respective cultures. All the plants used were large and fruitful, with heavy thick leaves, and they showed a fairly large loss of water over daily periods.

Miller (*23*) and Childers (*6*) were apparently the first two investigators to attempt to determine the effect of bordeaux mixture on the rate of transpiration of mature tomato plants that were grown under conditions favorable to a high rate of transpiration. Both workers reported that bordeaux mixture either had no effect on or actually caused a reduction in the rate of transpiration. They did not attempt to determine the interaction between the effects of spray and other environmental variables (concurrently) on the rate of transpiration of their plants. The present studies confirm and extend the applicability of those results.

In tables 1 and 2 data are given that indicate definitely that copper phosphate-bentonite-lime mixture, when applied as a fungicide, caused a highly significant increase in the rate of transpiration of mature plants. This definite increase in rate of water expenditure occurred regardless of how the increase was calculated. It is of special interest that there was no significant interaction between effect of spray and the other environmental variants. The response to spraying was consistent regardless of the cultural conditions. The data on total water transpired and water requirement basis also

indicated a definite increase in water utilization after the application of copper phosphate-bentonite spray. It has not been determined whether copper phosphate or bentonite or both substances were responsible for the results observed.

Very little is known about the effects of bentonite. This fact should be kept in mind before too definite conclusions are attempted with reference to the effect of copper phosphate on transpiration. The zinc-lime and bordeaux mixtures did not contain bentonite.

SUMMARY

In this report data are presented that show the effect of copper phosphate-bentonite-lime, zinc-lime, and bordeaux mixture on the rate of transpiration of large, fruiting tomato plants (*Lycopersicon esculentum* Mill.). Extensive data are also presented which show the lack of interaction of these fungicides under widely different environmental conditions on the rate of transpiration of tomato plants, and indicate that environment does not influence the effect of the spray mixtures on the transpiration.

Copper phosphate-bentonite-lime, applied as a fungicide, caused a significant increase in the rate of transpiration of mature tomato plants.

Zinc-lime and bordeaux mixture had no significant effect on the rate of transpiration under the experimental conditions reported.

Added increments of soil nitrogen, reduced soil moisture, and reduced air temperature all caused a marked reduction in rate of transpiration.

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THE CHLOROFORM-SOLUBLE COMPONENTS OF BEET LEAFHOPPERS AS AN INDICATION OF THE DISTANCE THEY MOVE IN THE SPRING[1]

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INTRODUCTION

Determination of the spring breeding areas from which beet leafhoppers (*Eutettix tenellus* (Bak.)) found in a cultivated district have come has presented a difficult problem. The most common method has been to make sweep-net collections from host plants at points between the infested territory and the suspected breeding areas.[2] Various types of traps [3] [4] have also been used with fair success. The sex ratio of adults has proved useful in some localities, since female leafhoppers have been found to move farther from their breeding source than do males.[5] More recently the amonnt of reserve energy of the beet leafhopper, as measured by its chloroform-soluble components, has been studied by a method developed by the senior author;[6] and determinations on leafhoppers collected at various points along dispersal routes indicate that these components decrease during flight, and may therefore be used as a measure of the distance the leafhoppers have traveled from their breeding source.

This paper reports studies of the chloroform extractives of female beet leafhoppers following dispersals from the Colorado River drainage northeastward into western Utah in 1932 and from southern Arizona to Grand Valley, Colo., in 1933, both representing long-distance dispersals, and following a dispersal in southern Idaho in 1932, 1933, and 1934, representing more localized movements.

EXPERIMENTAL PROCEDURE

The insects were collected along tbe suspected routes by means of a sweep net, and were immediately killed with calcium cyanide. They were then placed between pieces of crepe paper in small pill boxes and shipped to the laboratory, where the sexes were separated and the females analyzed for chloroform-extractive content. Three determinations were made on each sample except in two instances, when the sample was too small and only two determinations could be made.

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LONG-DISTANCE MOVEMENTS

In 1932 beet leafhoppers were collected at various points in western Utah between St. George and Nephi, a distance of 205 miles (fig. 1).[7] St. George is at the extreme northern limit of the Nevada-Utah breeding area, and the other collections were made in areas infested by movement from this area. These collections were made May 17–19, shortly after a dispersal had taken place. It has been observed that no increase in chloroform extractives can be detected for 5 to 7 days

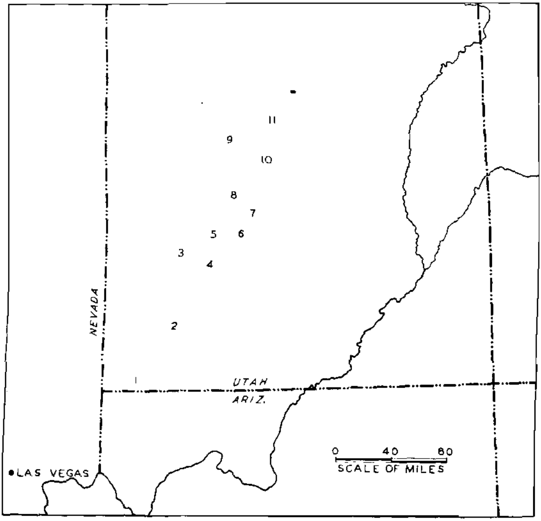

FIGURE 1.—Location of points where beet leafhoppers were taken along the western Utah dispersal routes during May 1932. Numbers refer to collection points designated in table 1.

after a dispersal. The sweep-net collections and the average chloroform-extractive contents, together with the distances from Las Vegas, Nev., a convenient point within the breeding area, are given in table 1.

Both the sweep-net collections and the chloroform extractives were in general greatest near the breeding area, and decreased progressively with the distance from the source. Collections 6 and 7 did not follow this trend, however, possibly because additional energy was required

[7] These data were collected by E. W. Davis, and the dispersal route is that shown by Dorst and Davis (see footnote 2).

in entering the valley over surrounding high mountains, or because many of the leafhoppers came from a more distant source or by a less direct route. The chloroform extractives of leafhoppers within the Utah, Nevada breeding area ranged between 38 and 46 percent; therefore, these determinations indicate that the source of these leafhoppers was quite distant.

TABLE 1.—*Sweep-net collections and the chloroform extractives of beet leafhoppers within the Nevada-Utah breeding area, and at various distances from this area, May 17–19, 1932*

Collection No.	Locality (Utah)	Airline distance from Las Vegas, Nev.	Mean leafhoppers per 100 sweeps		Chloroform extractives
			Adults	Nymphs	
		Miles	*Number*	*Number*	*Percent*
1	St. George	110	50.0	28.0	38.4
2	Cedar City	158	39.0	0	22.8
3	Milford	200	46.0	0	14.9
4	Beaver	206	18.0	0	15.7
5	Cove Fort	222	12.0	0	14.1
6	Sevier	238	22.7	0	8.7
7	Richfield	252	5.0	0	9.3
8	Fillmore	255	10.5	0	13.1
9	Lynndyl	286	11.0	0	11.1
10	Fayette	288	2.0	0	10.6
11	Nephi	315	4.0	0	8.9

In 1933 another set of samples for extraction was taken along a route between southern Arizona and the Grand Valley of Colorado (fig. 2), where survey work the previous year had given indications of leafhopper movements. At points along this route 18 collections were made from April 24 to 29, as soon as the first insects dispersed had reached the more northern points. The sweep-net and chloroform-extractives data for these collections are given in table 2.[8] The approximate distances were measured from Phoenix, Ariz., a convenient point in the breeding area.

TABLE 2.—*Sweep-net collections and the chloroform extractives of beet leafhoppers within the southern Arizona breeding areas and at intermediate points toward Grand Valley, Colo., April 24–29, 1933*

Collection No.	Locality	Airline distance from Phoenix, Ariz.	Mean leafhoppers per 100 sweeps		Chloroform extractives
			Adults	Nymphs	
		Miles	*Number*	*Number*	*Percent*
1	Marinette, Ariz	15	520.0	20.0	41.5
2	4 miles southeast of Wittmann, Ariz	35	156.0	24.0	42.4
3	Morristown, Ariz	43	8.3	3.0	46.3
4	2.4 miles south of Wickenburg, Ariz	52	68.0	28.0	39.5
5	3.2 miles south of Paulden, Ariz	105	82.4	0	28.4
6	Ashfork, Ariz	130	24.8	0	29.4
7	Seligman, Ariz	145	2.5	0	28.9
8	2.8 miles southwest of Tuba City, Ariz	205	30.4	0	27.6
9	6.2 miles south of Cedar Ridge, Ariz	215	29.6	0	25.5
10	17.7 miles northeast of Tuba City, Ariz	217	10.4	0	26.3
11	Tonalea, Ariz	222	20.8	0	25.7
12	Kayenta, Ariz	255	6.4	0	24.8
13	2.2 miles northeast of Mexican Hat, Utah	300	4.8	0	23.1
14	Blanding, Utah	335	4.0	0	22.6
15	Notum, Utah	350	.6	0	19.6
16	Hanksville, Utah	362	1.3	0	17.8
17	5.5 miles east of Thompsons, Utah	415	2.0	0	15.4
18	Grand Valley, Colo	450	.7	0	6.6

[8] *Some of the data shown in table 2 were collected by W. A. Shands, who was at that time in charge of the* Grand Junction, Colo., field laboratory of this Bureau.

The first four collections were made on different host plants withi the breeding source, and the average chloroform-extractive conten was 42.4 percent. Between collections 6 and 17 there was a fairl regular decrease in chloroform extractives with the increase in th distance from the breeding source. Collection 18 seems to be low

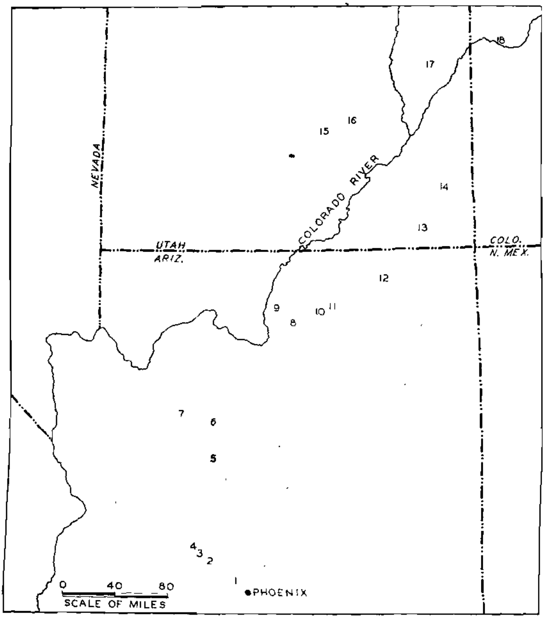

FIGURE 2.—Location of collections of beet leafhoppers taken along the Colorado drainage route during April 1933. Numbers refer to collection points designated in table 2.

since the drop from 15.4 to 6.6 percent in extractives within a distance of 35 miles seems out of line. Collections 6 to 18 also indicate in a general way that the leafhoppers decrease in number as the distance from the breeding source increases, although the sweep-net data are not nearly so consistent as those for the chloroform extractives.

LOCALIZED MOVEMENTS

In southern Idaho the cultivated beet districts have been considered to be infested with leafhoppers from adjacent breeding areas,[9] and this belief has been substantiated by a study of the chloroform extractives of females collected immediately after dispersals into these cultivated districts over a 3-year period. The data for 1932 in table 3 show the extractives of females from 4 points within the breeding area and from 17 infested beet fields in the Twin Falls-Jerome district (fig. 3). The closeness between the figures for the breeding areas and the beet fields indicates that the local breeding areas were responsible

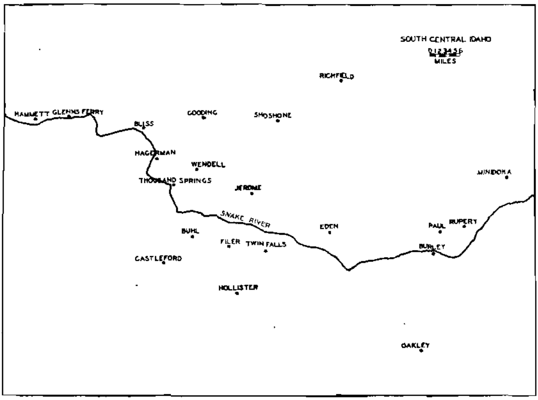

FIGURE 3.—Map of south-central Idaho showing towns near which collections were made in the desert breeding areas and in the beet fields.

for leafhoppers found in the southern Idaho beet fields following this movement.

The studies were repeated in the spring of 1933, collections being made at various localities in the western breeding areas, extending from Wendell to Glenns Ferry, Idaho, and also from the beet fields throughout the west end of the cultivated section. Collections in the east end were not taken at this time, inasmuch as the dispersing leafhoppers were extremely scarce and had not been detected east of Filer. The collections from the beet fields (Castleford, Buhl, and Wendell) were made as soon as the first influx of leafhoppers was detected (June 7–10). The results of the determinations are shown

[9] ANNAND, P. N., CHAMBERLIN, J. C., HENDERSON, C. F., and WATERS, H. A. MOVEMENTS OF THE BEET LEAFHOPPER IN 1930 IN SOUTHERN IDAHO. U. S. Dept. Agr. Cir. 244, 24 pp., illus. 1932.

in table 3. The slight decrease in chloroform-extractive content of the leafhoppers collected in the beet fields indicates a very near source for the 1933 infestation.

TABLE 3.—*Chloroform extractives of female beet leafhoppers collected shortly after the main influx into the beet-growing district in southern Idaho in 1932, 1933, and 1934*

Location of collection	Chloroform extractives from collections		
	1932	1933	1934
IN BREEDING AREA	*Percent*	*Percent*	*Percent*
2 miles south 1 mile west of Hagerman	38.6		
Thousand Springs	38.4		20.3
5.5 miles south of Thousand Springs	38.5		
4 miles north 7 miles west of Hollister	38.4		
1 mile west of Hammett			29.9
2 miles east of Hammett		34.7	
Glenns Ferry			26.4
2.2 miles west of Glenns Ferry		35.1	29.1
3 miles east of Glenns Ferry		34.6	
1 mile north 7 miles west of Castleford			26.3
7 miles west of Castleford		35.6	
1 mile south 4 miles west of Bliss		35.3	
Bliss			21.9
Average	38.5	35.1	25.7
IN BEET FIELDS			
1 mile south 1 mile west of Castleford	38.4		
3 miles south of Castleford	38.4		
4 miles south ¼ mile west of Castleford			17.1
5 miles east of Castleford	38.3		
1 mile east of Buhl		33.5	
1.5 miles east of Buhl	38.5		
4.5 miles east of Buhl	38.3		
3 miles north of Buhl	38.2	33.9	
2 miles south 4 miles west of Twin Falls	37.7		
2 miles west 2 miles north of Twin Falls	38.0		
4 miles west of Twin Falls	37.7		
Eden	36.9		
2 miles south 1 mile west of Burley	37.4		
2 miles west of Jerome		34.2	18.9
4 miles north 2 miles east of Jerome	38.3		
4 miles west 2 miles south of Jerome		33.7	
4.5 miles west of Jerome	38.4		
0.5 mile north of Paul	37.5		
4 miles east 2 miles south of Jerome	37.2		
8 miles north of Oakley	38.2		
1 mile west 2 miles north of Oakley	37.9		
1 mile south 2.5 miles east of Wendell			19.6
1 mile south 3 miles west of Wendell		34.1	
2 miles south ¼ mile west of Gooding			21.4
2 miles east 2¼ miles south of Jerome			17.8
5 miles south 3 miles east of Buhl			17.3
0.5 mile north of Filer			16.9
Average	38.0	33.9	18.4

Collections from Thousand Springs to Hammett and also from the western section of the beet-growing district were studied in the spring of 1934. These collections were made on May 10–11, about 2 weeks after the beginning of the movement and prior to its peak. The chloroform extractives found in these leafhoppers are given in table 3. The same relationship between the extractive content of leafhoppers taken in the breeding area and that of leafhoppers in the beet fields was maintained in that year, but both were decidedly lower than in 1932 and 1933. Either host-plant conditions or some unknown

factors caused the leafhoppers in the breeding area to have a comparatively low extractive content, or leafhoppers from a more distant source infested both the breeding areas and the beet fields.

CONCLUSION

There appears to be a steady reduction in the chloroform-soluble material of the leafhoppers as they move along an extended migration route away from the breeding area, but only a small variation where localized or short-distance flights occur. It seems possible, therefore, by extraction of individuals collected over sufficient area during a flight of insects to determine whether it is a local or a long-distance dispersion.

SUMMARY

The chloroform-soluble components of the beet leafhopper (*Eutettix tenellus* (Bak.)) have been studied as a means of determining the distance leafhoppers found in cultivated fields have traveled from their breeding source. The beet leafhoppers were collected at various points along dispersal routes and analyzed by a method developed by the senior author.

In 1932 the collections were made along a known long-distance dispersal route from the Colorado River drainage in Nevada northeastward into western Utah, and in 1933 along another suspected route from southern Arizona to Grand Valley, Colo. In 1932, 1933, and 1934 collections were also made in southern Idaho, where more localized movements were suspected.

Along the first route the percentage of total extractives decreased from 38.4 in leafhoppers taken at the point nearest the breeding source to 8.9 in leafhoppers taken at the farthest point, 205 miles away, and along the second route the percentage decreased from 39.5 to 6.6 over a distance of 398 miles. In southern Idaho the chloroform extractives of leafhoppers collected in the breeding areas were 38.5, 35.1, and 25.7 percent for the 3 years, respectively, as compared with 38.0, 33.9, and 18.4 percent in the beet fields. The closeness of the figures for the two areas indicates that the beet-growing districts of southern Idaho are infested principally from adjacent breeding grounds.

The chloroform extractives show a more consistent decrease as distance from the breeding source increases than do sweep-net counts of the insects, although the data corroborate each other. It seems possible, therefore, to distinguish between long-distance and local dispersions by determining the chloroform extractives of the leafhoppers collected along the known or suspected dispersal routes.

INHERITANCE OF SEED-COAT COLOR IN PEANUTS [1]

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INTRODUCTION

The principal seed-coat colors in the peanut (*Arachis hypogaea* L.) are due to water-soluble pigment or pigments, and the colors fade rapidly from mature peanuts left in moist soil and more slowly from those in dry storage. Frequently, seed from a single plant may show a wide range in intensity or shade of color because of fading and differences in stage of maturity; so that much confusion in regard to color nomenclature is found in the literature.

MATERIALS AND METHODS

The present discussion is based upon records of general characteristics of some 85 varieties and strains and of hybrid stocks developed from crosses among 16 of these varieties. In recording testa color, the color standards of Société Francaise des Chrysanthémistes (*4*)[2] have been found most convenient, as the relationship of the various tones and shades could be demonstrated. By the use of these standards the testa colors of the varieties and strains were separated readily into three color groups, red, flesh, and white. However, a pure self-color is rare in the harvested seed of any variety.

In the flesh-colored varieties the base color is usually salmon flesh, but it may vary from pale flesh to dark flesh. Usually pale reddish-lilac markings are found about the hilum end and along the veins and may spread as a flush over a large part of the seed, as it does typically in the Virginia Runner and the Virginia Bunch varieties. Occasionally, a violet flush may show about the tips of a few seeds or, rarely, may spread over the entire surface. This violet flush is most common on the seed from small pods, "nubbins" of the shellers, produced in the crown of the plant and more or less exposed to light. This exposure may account for the unusual pigmentation.

Of the varieties commonly grown in this country, the Spanish, North Carolina Runner (African), Virginia Runner, and Virginia Bunch are classed as "flesh" in the writer's grouping. Hull (*2*) found a genetic difference between the "russet" of Virginia Bunch and the "tan" of a Spanish strain. There are various shades of flesh, when compared with color standards, within the Spanish variety; but all appear to segregate similarly when crossed with a variety from the red or white color group. Intensity of flesh color and the reddish-lilac flush about the tip are undoubtedly inherited characteristics; but the genetic factors producing them appear to be separate from the factors responsible for the base color, and for the purpose of color grouping all these various shades are here classed as flesh.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 752.

The reds place typically about salmon lilac or Indian lake of the color standards; but seed from a single plant may range from pale reddish lilac through reddish lilac, salmon lilac, or Indian lake to vinous purple or even slate violet. When mature red seed are wet, the color usually fades to slate violet. In a few hybrid strains the color is typically slate violet, but these are classed with the reds because this color appears to be common to all red varieties. Vinous purple appears to bear the same relation to red seed as does violet to flesh colored. The purple is most pronounced about the hilum tip, apparently the result of a mixture of violet and red pigments. Neither in the writer's variety collection nor among his hybrid stocks have any been found in which all seeds were vinous purple.

Patel, John, and Seshadri (*5*) have recently reported the genetics of several seed-coat colors, including a "dark purple" as found in the Corientes–3 variety. They found purple testa dominant to red, "rose" (flesh), and white; that Corientes–3 carries the factor for "rose"; and that "rose" is necessary for the expression of purple. This variety is not included in the writer's collection.

The variety collection used in the present study includes only two white-seeded varieties: Philippine White, with greenish-white seed coats which weather to yellowish-white; and the Pearl variety, with seed coats usually "lilacy" white, also weathering to yellowish-white. Among the white-seeded hybrid strains the colors range through snow white, sky-colored white, purplish-tinted white, "lilacy" white, milk-white, yellowish-white, greenish-white, and fleshy-white. The last-named may sometimes, by casual observation, be classed as pale flesh, but otherwise the whites are easily separated from the other color groups.

The Pearl variety came to the writer in 1931 in a collection of peanut varieties from a commercial firm with the notation that it was obtained from a farmer near Opelika, Ala. The vine type resembles the Spanish variety very closely, but certain characteristics suggest those of the Valencia variety, which is grown to some extent throughout the South, principally as a roasting nut for home consumption. The characteristics of the Pearl variety suggest that it may have originated as a chance cross between the Valencia and the Spanish varieties. So far the writer has been unable to trace the variety to its original source, and any statement as to origin must be accepted as a tentative guess.

EXPERIMENTAL RESULTS

The results obtained from crossing the Philippine White variety with three varieties (Spanish, Virginia Runner, and North Carolina Runner) having flesh-colored testa are shown in table 1. The genetic constitution of the varieties with flesh-colored testa are indicated by $F_1F_1F_2F_2$ and that of Philippine White by $f_1f_1f_2f_2$. In all three crosses the seed of F_1 plants was flesh and that of F_2 flesh and white in a 15:1 ratio. These results indicate that the three flesh-colored varieties have duplicate genes for flesh color and thus confirm the results reported by Patel, John, and Seshadri (*5*) from crosses (Philippine White × Saloum and Philippine White × Gudiyattam) involving the same colors.

TABLE 1.—*Inheritance of flesh-colored testa*

Parents		F_2 phenotypes [1]		χ^2	P
Flesh $F_1F_1F_2F_2$	White $f_1f_1f_2f_2$	*Flesh*	*White*		
Spanish, I. W. × Philippine White:					
Observed distribution		73	5		
Expected 15:1 ratio		73	5	0.0034	0.95+
Virginia Runner × Philippine White:					
Observed distribution		61	5		
Expected 15:1 ratio		62	4	.1980	.7−
North Carolina Runner × Philippine White:					
Observed distribution		43	2		
Expected 15:1 ratio		42	3	.2504	.7−
Total observed		177	12		
Expected 15:1 ratio		177	12	.0032	.95+

[1] F_1 phenotype, all flesh.

The results obtained from crosses involving three varieties with red and seven with flesh-colored testa are shown in table 2. In every case red is dominant to flesh, with a single factor difference, confirming results reported by Van der Stok (*6*), Hayes (*1*), Stokes and Hull (*7*), and Patel and his coworkers (*5*).[3] The results are given here principally for comparison with crosses involving Pearl.

TABLE 2.—*Inheritance of red testa*

Parents		F_2 phenotypes [1]		χ^2	P
Red $RRF_1F_1F_2F_2$	Flesh $rrF_1F_1F_2F_2$	Red	Flesh		
Tennessee Red × Virginia Runner:					
Observed distribution		28	9		
Expected 3:1 ratio		28	9	0.0090	0.9+
Tennessee Red × Virginia Bunch:					
Observed distribution		38	14		
Expected 3:1 ratio		39	13	.1026	.7+
Tennessee Red × North Carolina Runner:					
Observed distribution		96	32		
Expected 3:1 ratio		96	32	.0000	1.0
Tennessee Red × West African:					
Observed distribution		54	18		
Expected 3:1 ratio		54	18	.0000	1.0
Tennessee Red × Spanish, I. W.:					
Observed distribution		68	20		
Expected 3:1 ratio		66	22	.2424	.7−
Kimorales × Spanish, No. 167:					
Observed distribution		96	26		
Expected 3:1 ratio		92	30	.8852	.3+
Kimorales × Java P. L.:					
Observed distribution		90	33		
Expected 3:1 ratio		92	31	.2195	.7−
Valencia × H210:					
Observed distribution		18	7		
Expected 3:1 ratio		19	6	.1200	.7+
Total observed distribution		488	159		
Expected 3:1 ratio		485	162	.0624	.8+

[1] F_1 phenotype, all red.

[3] Also V. K. Badami in unpublished thesis, Cambridge University, to which reference was made by Hunter and Leake (*3*, pp. 339-341).

In every cross involving the Pearl variety, except certain backcrosses, the F_1 seeds have red testa. Results from all crosses shown in table 3 indicate that Pearl carries factors for both red and flesh pigments but either lacks factors for expression of color or carries factors that inhibit the development of color. Until we know more about the chemical nature of peanut pigments, it would seem most logical to assume the absence of factors for development of color. With this assumption we must postulate two factors to explain the 15:1 ratio of reds and whites observed in the crosses Pearl × Tennessee Red and Pearl × Small Japan; whereas the 3:1 ratio obtained in the cross Pearl × Valencia would indicate a single factor difference and that the Valencia variety lacks one factor for development of color.

TABLE 3.—*Inheritance of testa color in crosses involving the Pearl variety*

Parents (variety, composition, and testa color)	F_2 phenotypes [1] Red	Flesh	White	x^2	P
Pearl $RRF_1F_1F_2F_2d_1d_1d_2d_2$ (white) × Philippine White $rrf_1f_1f_2f_2D_1D_1D_2D_2$ (white):					
Observed distribution	98	16	5		
Expected 675:225:124 ratio	79	26	14	15.0121	0.01−
Expected 720:225:79 ratio	84	26	9	8.2973	.02−
Pearl $RRF_1F_1F_2F_2d_1d_1d_2d_2$ (white) × Virginia Runner $rrF_1F_1F_2F_2D_1D_1D_2D_2$ (flesh):					
Observed distribution	338	89	26		
Expected 45:15:4 ratio	319	106	28	4.1566	.1+
Pearl $RRF_1F_1F_2F_2d_1d_1d_2d_2$ (white) × North Carolina Runner $rrF_1F_1F_2F_2D_1D_1D_2D_2$ (flesh):					
Observed distribution	366	108	29		
Expected 45:15:4 ratio	354	118	31	1.4489	.5−
Total observed distribution	704	197	55		
Expected 45:15:4 ratio	672	224	60	5.1530	.1−
Pearl $RRF_1F_1F_2F_2d_1d_1d_2d_2$ (white) × Tennessee Red $RRF_1F_1F_2F_2D_1D_1D_2D_2$ (red):					
Observed distribution	44		3		
Expected 15:1 ratio	44		3	.0015	.95+
Pearl $RRF_1F_1F_2F_2d_1d_1d_2d_2$ (white) × Small Japan $RRF_1F_1F_2F_2D_1D_1D_2D_2$ (red):					
Observed distribution	401		33		
Expected 15:1 ratio	407		27	1.3573	.2+
Pearl $RRF_1F_1F_2F_2d_1d_1d_2d_2$ (white) × Valencia $RRF_1F_1F_2F_2D_1D_1d_2d_2$ (red):					
Observed distribution	42		16		
Expected 3:1 ratio	44		14	.2069	.7−

[1] F_1 phenotype, all red.

From the results obtained in crossing Philippine White with several other varieties, including those with purple, red, and "rose" (flesh) seed coats, Patel et al. (*5*) reached the conclusion that the Philippine White variety carries no factor for seed coat color; also that the presence of "rose" (flesh) pigment is necessary for the expression of either red or purple color. The results from crossing this variety with Pearl are, therefore, of special interest. In the crosses Pearl×Philippine White and reciprocal, the testa of the zygote is white, the shade of the pistillate parent, that of seed from F_1 plants is red, Indian lake to vinous purple, and that from F_2 seed red, flesh, and white. While the Philippine White variety lacks both red and flesh pigment,

ties, apparently, carry factors for development of color, and we may express the genetic constitution as $rrf_1f_1f_2f_2D_1D_1D_2D_2$ and that of the Pearl variety as $RRF_1F_1F_2F_2d_1d_1d_2d_2$. Assuming the presence of a flesh factor necessary for expression of red, the expected ratio would be 675:225:124, red, flesh, and white, respectively. If flesh pigment is not necessary for the expression of red, the ratio would be 720:225:79. The observed ratio agrees somewhat better with the latter assumption, yet other evidence for the assumption that flesh is necessary for the development of red color is so strong that it seems safer to explain otherwise the poor agreement of the observed with the expected ratio.

The total number of progenies observed is too small to give a very close approximation of the expected ratio when so many factors are involved. Furthermore, the Philippine White variety produces, at least under the conditions of the present study, a very high percentage of chaffy, nonviable seed. In the hybrid stocks this characteristic is accentuated, many plants producing no seed at all. A lethal (albino) factor is also involved in this cross, but the records do not justify any attempt to correlate this factor with the ratios obtained. However, the fact that reds are in excess in this cross and also in the cross Pearl×Virginia Runner suggests the possible correlation of lack of pigment with unfruitfulness and with nonviability of seed.

In the F_3 and succeeding generations various red phenotypes were observed, producing all red, red and flesh, red and white, and red, flesh, and white in various ratios; but the number of F_3 families was too small to include all genotypes. The flesh bred true or gave flesh and white. The whites always bred true.

If the assumed genetic constitution of the Pearl and the Philippine White varieties is correct, we should expect two classes of whites in the F_2 and succeeding progenies; i. e., those with factors for pigmentation but lacking factors for development of color and those lacking factors for pigment.

Results obtained with one red-seeded F_2 selection, H220–13, from this cross indicates the presence of the two classes of whites and also gives very substantial evidence that the presence of the flesh pigment factor is necessary for development of red color. In this selection the seed of the F_2 plant was red. In the F_3, 48 plants produced red seed and 18 produced white, none flesh. Plants from each of the white sibs were backcrossed to Pearl and the same plants crossed also with North Carolina Runner. Unfortunately, mice destroyed many of the seeds before maturity. However, seed was obtained from 13 sibs with Pearl and from 9 with North Carolina Runner. All the crosses with North Carolina Runner produced red seed in the F_1 generation, indicating that all these whites carried the factor for red pigment. Eight of the 13 sibs when crossed with Pearl also produced one or more F_1 plants with red seed, indicating that they carried at least one factor for development of color.

From the assumed genetic constitution of the parents there are three possible genotypes that would approximate the 48 red and 18 white segregation of H220–13.

		Red	White		
1	$RRF_1F_1F_2F_2D_1d_1d_2d_2$ or $RRF_1F_1F_2F_2d_1d_1D_2d_2$	3	1	all whites like Pearl.	
2	$RRF_1f_1F_2f_2D_1d_1d_2d_2$ or $RRF_1f_1F_2f_2d_1d_1D_2d_2$	45 or 3	19 1	3 whites not like Pearl. assuming red independent of flesh.	
3	$RRF_1f_1f_2f_2D_1d_1D_2d_2$ or $RRf_1f_1F_2f_2D_1d_1D_2d_2$	45 or 15	19 1	15 whites not like Pearl. assuming red independent.	
Observed distribution		48	18	χ^2	P
Expected 3:1 ratio		49.5	16.5	0.1818	0.7–
Expected 45:19 ratio		46.4	19.6	.1901	.7–

The observed ratio is evidently not near 15:1 under proposition 3. The 3:1 ratio gives a slightly lower χ^2 value than the 45:19 ratio. but in both cases where the 3:1 ratio is obtained all the whites are like Pearl, lacking factors for expression of color, and could not give red testa when crossed with Pearl. Since all produced red seed coats when crossed with North Carolina Runner and more than half of the sibs gave red when crossed with Pearl, we are forced to the assumption that the factor for red was present in all but in some the color did not develop because of the absence of the developer factor and in others because of the absence of flesh pigment. The results also indicate that presence of the flesh factor is necessary for expression of red color and that the genetic constitution of H220–13 was either $RRF_1f_1f_2f_2D_1d_1D_2d_2$ or $RRf_1f_1F_2f_2D_1d_1D_2d_2$.

In the crosses between Pearl and the varieties with flesh seed coats the above white genotype lacking factors for flesh pigment would not be obtained. All whites would be homozygous for flesh pigment, and three fourths would have the factor for red; but all would lack factors for color development. Along with the above series of crosses seven white-seeded selections from the cross Pearl × Virginia Runner were backcrossed to Pearl and four of these were also crossed with North Carolina Runner. In all seven crosses with Pearl the F_1 progenies produced white seed and all four crossed with North Carolina Runner produced red seed in F_1, indicating that the selections were of the Pearl genotype.

Records of seed-coat color were obtained for 118 F_3 families from the Pearl × Virginia Runner and Pearl × North Carolina Runner crosses. As shown in table 4, this number apparently included all possible genotypes. Approximately half the recorded families were selected because of resistance to disease, high yield, and other characteristics desirable for a peanut variety. For this reason, there was conscious preference for strains with flesh-colored seed coats; yet the observed distribution of families among the various genotypes is in fairly close agreement with the expected: $\chi^2=11.2149$, $P=0.3$.

LE 4.—*F_2 genotypes and F_3 distribution of phenotypes from crosses Pearl × Virginia Runner and Pearl × North Carolina Runner*

um- in 4	F_2 genotypes	Color of F_2	Number of families observed	F_3 distribution			χ^2	P
				Red	Flesh	White		
1	$RRF_1F_1F_2F_2D_1D_1D_2D_2$	Red						
2	$RRF_1F_1F_2F_2D_1D_1D_2d_2$							
2	$RRF_1F_1F_2F_2D_1d_1D_2D_2$		14	(1)				
1	$RRF_1F_1F_2F_2D_1D_1d_2d_2$							
1	$RRF_1F_1F_2F_2d_1d_1D_2D_2$							
4	$RRF_1F_1F_2F_2D_1d_1D_2d_2$	do	11	15 670		1 50	0.5926	0.5–
2	$RRF_1F_1F_2F_2D_1d_1d_2d_2$	do	6	3 286		1 86	.7025	.5–
2	$RRF_1F_1F_2F_2d_1d_1D_2d_2$							
2	$RrF_1F_1F_2F_2D_1D_1D_2D_2$	do	25	3 1,266	1 399		.9532	.3
4	$RrF_1F_1F_2F_2D_1D_1D_2d_2$							
2	$RrF_1F_2F_2D_1D_1d_2d_2$							
2	$RrF_1F_1F_2F_2d_1d_1D_2D_2$							
8	$RrF_1F_1F_2F_2D_1d_1D_2d_2$	do	13	45 698	15 251	4 68	1.3880	.5
4	$RrF_1F_1F_2F_2D_1d_1d_2d_2$	do	7	9 348	3 105	4 145	.9900	.7–
4	$RrF_1F_1F_2F_2d_1d_1D_2d_2$							
1	$rrF_1F_1F_2F_2D_1D_1D_2D_2$	Flesh	14		(1)			
2	$rrF_1F_1F_2F_2D_1D_1D_2d_2$							
2	$rrF_1F_1F_2F_2F_1d_1D_2D_2$							
1	$rrF_1F_1F_2F_2D_1D_1d_2d_2$							
1	$rrF_1F_1F_2F_2d_1d_1D_2D_2$							
4	$rrF_1F_1F_2F_2D_1d_1D_2d_2$	do	6		15 289	1 18	.0784	.8–
2	$rrF_1F_1F_2F_2D_1d_1d_2d_2$	do	13		3 604	1 202	.0017	.95+
2	$rrF_1F_1F_2F_2d_1d_1D_2d_2$							
1	$RRF_1F_1F_2F_2d_1d_1d_2d_2$	White	8			(1)		
2	$RrF_1F_1F_2F_2d_1d_1d_2d_2$							
1	$rrF_1F_1F_2F_2d_1d_1d_2d_2$							

[1] All.

SUMMARY AND CONCLUSIONS

By the use of color standards the testa color of 85 varieties and :rains of peanuts, as well as of hybrid progenies from crosses among 1ese varieties, were separated into three color groups, red, flesh, and hite. A fourth color, dark purple, as reported by Patel et al. (*5*) ɔr the Corientes-3 variety, is not included in this study.

Flesh-colored testa is dominant to genetically pure white with a igenic difference, indicating that all the flesh-colored varieties so far tudied, both bunch and runner types, have two identical genes for olor.

Red testa is dominant to flesh with a single factor difference, but he flesh pigment is necessary for expression of red color.

Two varieties with white seed coats were found to differ in their enetic constitution. Apparently the Pearl variety carries factors ɔr both red and flesh pigment but lacks factors for expression of olor, while the Philippine White has neither pigment but does carry actors for development of color.

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EFFECTIVENESS OF HEAT PENETRATION IN MEAT CANNED IN GLASS JARS IN A PRESSURE COOKER[1]

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INTRODUCTION

It has previously been shown[2] that under conditions prevailing when meat is processed in sealed tin containers and sterilized in a retort under steam pressure the effectiveness of heat penetration and the sterilizing process depend upon five variables; namely, type of pack, amount of steam pressure, size of can, time of processing, and method of cooling. Heat penetration is most rapid when there is least interference with convection currents. Fifteen pounds steam pressure is necessary to obtain sterility in the strict sense of the laboratory. The period required to obtain sterility varies directly with the size of the container.

Other factors being constant and controllable, the type of container used in the home canning of meat may be the factor determining the success of the process. Since glass jars are the containers commonly used in the home and since the rate of heat transference is slower through glass than through tin, this investigation was undertaken to determine the length of the processing period necessary in canning a solid pack of meat in glass jars.

EQUIPMENT AND PROCEDURE

In order that the temperature might be recorded at regular interval throughout the processing period, a 12-quart pressure cooker was modified in the manner first described by Magoon and Culpepper[3] and later by Nelson and Berrigan.[4]

Wide-mouthed, quart-size, glass jars with the mason-type Kerr lids were used to facilitate the soldering of the brass plate to the jar lid.

It was necessary to use a long-stemmed thermometer on which the graduation started 6 inches from the mercury bulb in order that readings might be visible above the top of the retort cover.

The top cut of beef round was used throughout this study. The eye muscle was first sectioned out, and then the rest of the beef was cut into pieces of a size that could be conveniently placed in the jar.

Test cultures of bacteria were sealed in thin-walled, glass ampoules and buried deep in the eye muscle, since previous work[4] had shown this to be a reliable method. This section of the round was then wrapped in a single layer of cheesecloth in order that it might be quickly identified after the processing period.

A glass jar was half filled with meat, the eye muscle containing the test organisms placed in the center, and the remaining space packed

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[4] See footnote 2.

with portions of the beef round. To maintain as nearly equivalent conditions as possible, the contents of all jars were adjusted to approximately the same weight.

After the jars were packed, they were immersed in boiling water and preheated until the temperature of the center of the pack was 30° C.

Since it had previously been reported [5] that jars may be sealed tight before processing in a pressure cooker, the jar lid which had been soldered to the retort equipment described above was screwed firmly onto the jar. By this arrangement the jar was suspended exactly in the center of the retort. A layer of water about 1 inch deep was maintained in the pressure cooker. After the cooker had been sealed, the pet cock was left open until the air escaped. After the steam pressure had reached 15 pounds, the temperature was recorded at 1-minute intervals. At the end of the processing period the jars were removed from the pressure cooker and allowed to cool to room temperature. Since cooling could not be hastened by immersion of the jars in cold water, as is the usual procedure when tin cans are used, the total sterilizing effect of the processing was prolonged. This was especially marked in the case of the storage jars, since they were tightly sealed before processing and a greater length of time was required to cool them. Usually the broth in these jars boiled for 3 to 5 minutes after the jars were removed from the pressure cooker.

The bacterial cultures used to test the sterilizing process were *Clostridium botulinum*, Type A (in an aged-spore state), *Escherichia* *coli* (fresh suspension), and *Bacillus mesentericus* (aged-spore state). The bacterial suspensions were prepared in physiological saline solution. Although such a saline solution has been shown to have the effect of reducing the viable count of a bacterial suspension on standing it was not considered that the heavy suspension used would be weakened to the extent of invalidating the results obtained. These suspensions were maintained at uniform turbidity throughout the experiment.

Ampoules from all jars were removed under aseptic conditions and the contents placed in appropriate culture media. Robertson's heart medium was used for culturing *Clostridium botulinum*, lactose fermentation tubes for *Escherichia coli*, and nutrient broth for *Bacillus* *mesentericus*. All tubes were incubated at 37° C. for a week. Growths were identified by appropriate criteria.

A total of 38 jars of meat were prepared in the manner described, and these were processed over periods of 50, 60, 65, 70, 80, 90, 100, 110, and 120 minutes. At least four jars were prepared at each interval of time; half were opened immediately for bacteriological examination and the other half were stored in an incubator at 25° C. for 6 months.

EXPERIMENTAL DATA

In figures 1 to 4 are depicted curves obtained by plotting time of processing against temperature. The temperature curves shown in figures 1 and 2 were drawn from temperature readings made with an ordinary stock thermometer. Variation was pronounced. Figures 3 and 4 represent the continous use of one especially accurate thermometer. These curves well illustrate the difficulty of checking all

[5] FELLERS, C. R., MACLINN, W. A., and LEVINE, A. S. HOME CANNING RESEARCH. Mass. Agr. Expt. Sta. Bul. 331: 70-71. 1937.

variable factors. The distribution of fat and lean affects the rate of heat penetration. Although all the fat was removed that could be removed, some pieces of the meat contained decidedly more interstitial fat than others. The difficulty of limiting all the variable factors in a study of this type has been clearly pointed out by Lang.[6] A beef pack, however, does not offer such an opportunity for change in

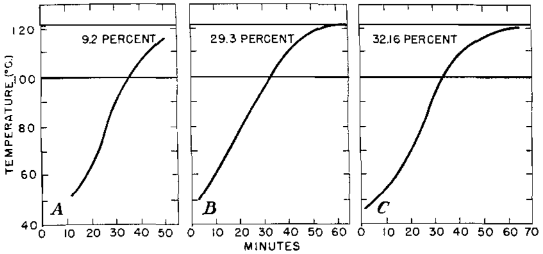

FIGURE 1.—Variations in effective heat penetration (see text for fuller explanation) as affected by length of processing period. Processing periods: *A*, 50 minutes; *B*, 60 minutes; *C*, 65 minutes.

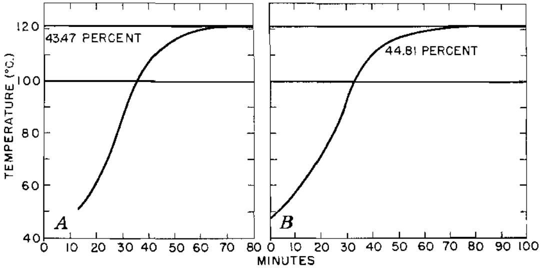

FIGURE 2.—Variations in effective heat penetration (see text for fuller explanation) as affected by length of processing period. Processing periods: *A*, 70 minutes; *B*, 80 minutes.

the fluidity of the pack during processing as do the softer marine foods, and it may safely be assumed that curves will not be broken out of their regularity by major changes in heat convection.

The space between the two heavy horizontal lines on the graphs, drawn at 100° and 120° C., indicates the zone of sterilization. The curves shown were originally produced from temperature data plotted on millimeter squared ruling. Each curve circumscribes a dome-

[6] LANG, O. W. THERMAL PROCESSES FOR CANNED MARINE PRODUCTS. Calif. Univ. Pubs., Pub. Health 182 pp., illus. 1936.

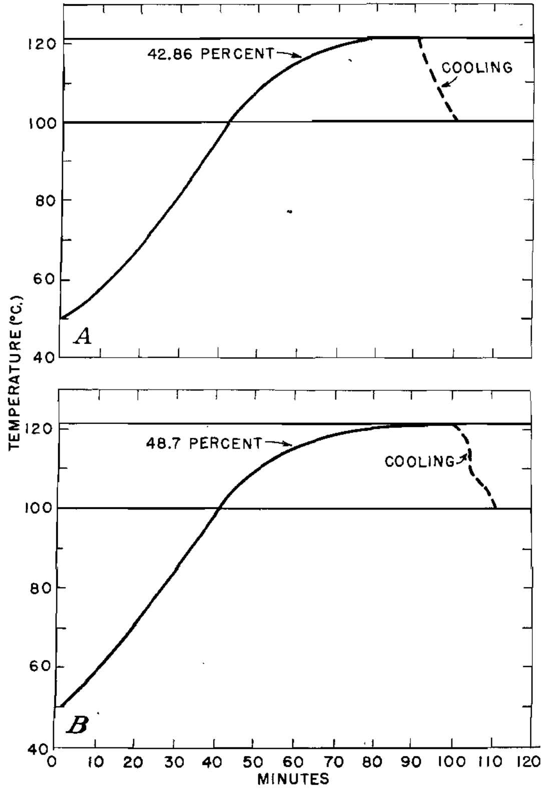

FIGURE 3.—Variations in effective heat penetration (see text for fuller explanation) as affected by length of processing period. Processing periods: *A*, 90 minutes; *B*, 100 minutes.

shaped area that represents sterilization accomplished as a product of time and temperature. The maximum sterilization possible would be represented by an area between the two horizontal lines circumscribed by a temperature curve rising abruptly to 121° at the

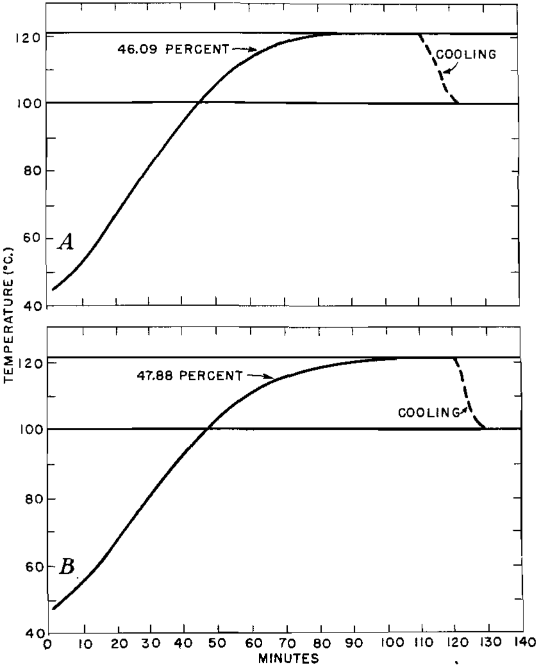

FIGURE 4.—Variations in effective heat penetration (see text for fuller explanation) as affected by length of processing period. Processing periods: *A*, 110 minutes; *B*, 120 minutes.

beginning of the processing. This is observed when water or broth is processed in a tin can.[7]

By accurately computing the area of the space circumscribed under the curve representing the experimental data of a particular processing period and comparing it with the area representing sterilization

[7] See reference given in footnote 2.

of water or broth, a ratio is obtained which expresses the efficiency of sterilization in terms of percent. (The reproduction of the curves in this paper may differ slightly from the curves originally plotted but the percentage holds true.) In figures 5, the averages of efficiencies of heat penetration for each processing period are shown in gradient. The curve reaches its crest at approximately the 90-minute period. This indicates that heat penetration reaches its practical maximum at 15 pounds steam pressure, when quart glass jars are used, in between 80 and 90 minutes after the beginning of the processing. Attainment of maximum efficient heat penetration is not necessarily identical with sterilization efficiency. Heat effective-

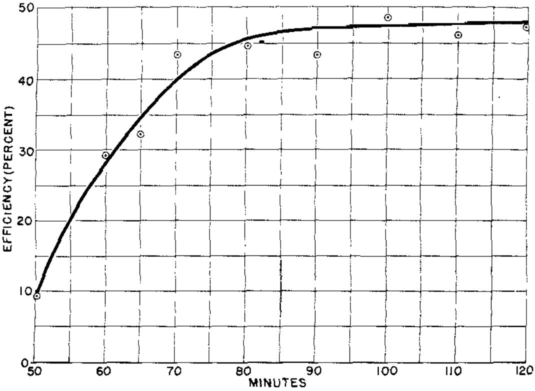

FIGURE 5.—Average percentage efficiency of heat penetration as affected by length of processing period.

ness in killing bacteria can best be determined by exposing the bacteria to the process over the same range of time and temperature used in determining heat penetration and efficiency.

The results obtained from the bacteriological examination of all processed jars are recorded in table 1. The examination of jars opened immediately after processing indicated that (1) the toxigenic anaerobe *Clostridium botulinum* in the spore state was destroyed by processing for 70 minutes at 15 pounds pressure; (2) *Escherichia coli*, a non-spore-forming organism, was destroyed by processing for 70 minutes at 15 pounds pressure (it appeared to be more susceptible to heat than *Clostridium botulinum*), and (3) the spore-bearing, aerobic, soil-inhabiting *Bacillus mesentericus*, in the spore state, was not destroyed during any period of processing tried, up to and including 120 minutes.

Table 1.—*Survival of test organisms in jars of meat processed at 15 pounds pressure*

Jars opened immediately					Jars stored				
Jar No.	Processing period	Survival of—			Jar No.	Processing period	Survival of—		
		Clostridium botulinum	*Escherichia coli*	*Bacillus mesentericus*			*Clostridium botulinum*	*Escherichia coli*	*Bacillus mesentericus*
	Minutes					*Minutes*			
4	50	[1] +	0	+	13	50	0	0	0
6	50	+	0	+	15	50	0	0	0
					3 [4]	60	0	0	+
[illegible]	60	0	0	+	5	60	0	0	0
[illegible]	60	0	0	+	7	60	0	0	0
10	65	+	[2] +	+	9	65	0	0	0
2	65	0	0	+	11	65	0	0	0
					17 [4]	70	0	0	0
8	70	0	0	+	19	70	0	0	0
20	70	0	0	+	37	70	0	0	0
22	80	0	[2] +	+	21	80	0	0	0
24	80	0	0	+	23	80	0	0	+
39	90	0	0	+	25	90	0	0	0
40	90	0	0	+	27	90	0	0	+
41	100	0	0	+	29	100	0	0	0
42	100	0	0	+	31	100	0	0	0
43	110	0	[2] +	+	38	110	0	0	0
44	110	0	0	0 (?)	47	110	0	0	
45	120	0	0	+	33 [3]	120	0	0	+
46	120	0	0	+	35	120	0	0	+

[1] Plus sign indicates survival.
[2] Indications of contamination. In these instances in which *E. coli* appeared to survive, the authors had excellent reason to believe that its presence was not due to survival but to experimental variance. The checks were free from growth. However, at extremes of ranges of viability, it is very possible that heat-resistant individuals might appear.
[3] Leaker.

An examination of the jars stored at 25° C. for 6 months or more showed that (1) *Clostridium botulinum* and *Escherichia coli* had been destroyed in all jars; (2) *Bacillus mesentericus* still persisted in a few. It has been pointed out before that the stored jars necessarily had a more prolonged cooling period, which no doubt increased the sterilizing effect of the processing.

SUMMARY AND CONCLUSION

The results obtained in this study indicate that the maximum point of efficiency in heat penetration in the processing of solid packs of beef in quart glass jars is reached at approximately 90 minutes. This represents a maximum efficiency of less than 50 percent.

The home canning of beef in quart glass jars in a pressure cooker apparently is safe if the pack is preheated to 30° C. and processed 90 minutes at 15 pounds steam pressure.

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RESPONSE OF TWO CLONAL STRAINS OF TRIUMPH POTATOES TO VARIOUS CONTROLLED ENVIRONMENTS[1]

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INTRODUCTION

In a previous publication the writer reported the response of one strain of Triumph potatoes to gradually changing photoperiod and temperature and to abrupt changes in photoperiod and nitrogen supply.[2] Further experiments have been conducted to determine whether other strains or varieties would respond in a similar manner to different environmental treatments. The environmental conditions under which tests were made involved different levels of nitrogen nutrition, constantly long or short days in contrast to gradually changing day lengths, and long or short days at several temperatures. Because of the difficulty of arriving at a satisfactory evaluation of a strain or variety by field tests [3] another objective was to determine whether varieties or strains can be analyzed as to their adaptation to various latitudes by testing them under controlled conditions with the photoperiods and at temperatures characteristic of certain latitudes.

EXPERIMENTAL METHODS

During the winters of 1934–35 and 1935–36 Triumph potatoes of two or three clonal strains were grown in the greenhouse with nutrient solutions in essentially the same manner as described in an earlier publication.[4] In 1935–36 the nitrogen-deficient treatment was provided by using only 10 percent as much nitrogen in the solution as was used in the complete solution. Six weeks after the plants emerged this was increased to 20 percent. These plants manifested very distinct nitrogen-deficiency symptoms. Photoperiods of specific duration were provided by 100-watt bulbs placed about 3½ feet apart and maintained about 2 to 2½ feet above the tops of the plants (fig. 1). All lights were turned on at 7 a. m. so as to avoid the variable light conditions incident to the beginning of midwinter days. Lights were turned off whenever daylight seemed brighter than the artificial light.

[1] Received for publication November 27, 1939. Paper No. 243 of the Journal Series of the Nebraska Agricultural Experiment Station.

[2] WERNER, H. O. THE EFFECT OF A CONTROLLED NITROGEN SUPPLY WITH DIFFERENT TEMPERATURES AND PHOTOPERIODS UPON THE DEVELOPMENT OF THE POTATO PLANT. Nebr. Agr. Expt. Sta. Res. Bul. 75, 32 pp., illus. 1934.

[3] WERNER, H. O. PERFORMANCE OF CLONAL STRAINS OF TRIUMPH POTATOES. I. TRIUMPH STRAINS ON DRY LAND IN WESTERN NEBRASKA. Amer. Potato Jour. 17: [66]–80. 1940.

——— *PERFORMANCE OF CLONAL STRAINS OF TRIUMPH POTATOES. II. COMPARISON OF STRAINS UNDER IRRIGATION CONDITIONS IN WESTERN NEBRASKA.* Amer. Potato Jour. 17: [95]–99. 1940.

——— PERFORMANCE OF CLONAL STRAINS OF TRIUMPH POTATOES. III. COMPARISON OF STRAINS IN CENTRAL NEBRASKA (AT NORTH PLATTE) WITH AND WITHOUT IRRIGATION. Amer. Potato Jour. 17: [123]–127. 1940.

——— PERFORMANCE OF CLONAL STRAINS OF TRIUMPH POTATOES. IV. COMPARISON OF TRIUMPH STRAINS IN EASTERN NEBRASKA (AT LINCOLN) WITH AND WITHOUT IRRIGATION. Amer. Potato Jour. 17: [153]–155. 1940.

——— PERFORMANCE OF CLONAL STRAINS OF TRIUMPH POTATOES. V. COMPARISON OF TRIUMPH STRAINS IN VARIOUS SOUTHERN STATES. Amer. Potato Jour. 17: [174]–181. 1940.

[4] See footnote 2.

In the late afternoon lights were turned on whenever artificial ligl was brighter than natural daylight. On dark days lights were le on throughout the day. Temperature was thermostatically coı trolled so that the desired mean was secured during each 24-hoı period. The day temperature was 10° to 20° F. higher than the nigl temperature. With "southern" or "northern" conditions phot periods and temperatures were changed once each week to simula changes that occur during the potato-growing seasons in the differeı

FIGURE 1.—Potato plants growing under "southern" conditions (S34). Phot graphed January 14, 1935.

latitudes. The general characteristics of the various treatments aı outlined in table 1. In 1934–35 the southern-day conditions weı similar to those that prevail in southern Louisiana and the northeı ones simulated a cool season in northwestern Nebraska (fig. 2).[5 6] I 1935–36 the southern-day conditions were similar to those of fa southern points, such as Brownsville, Tex., or Homestead, Fla., an the northern conditions resembled those of northwestern Nebrask in a warm season (fig. 3). The plants of one light or temperatuı treatment were necessarily grouped in one part of the greenhouse, bı plants harvested on each date of each strain within a treatment weı

[5] MARVIN, C. F. SUNSHINE TABLES. 3 v. 1905. Part I, Latitudes 20° to 30° North (reprinted), W. 805, 1923; Part III, Latitudes 40° to 50° North, W. B. 320, 1905.

[6] MARVIN, CHARLES F. NORMALS OF DAILY TEMPERATURE FOR THE UNITED STATES. U. S. Weath Bur. Monthly Weather Rev., Sup. 87 pp., illus. 1925.

distributed throughout the area so as to equalize place variations. When two nutrient solutions were used the plants that received the same solution were randomized throughout the greenhouse area.

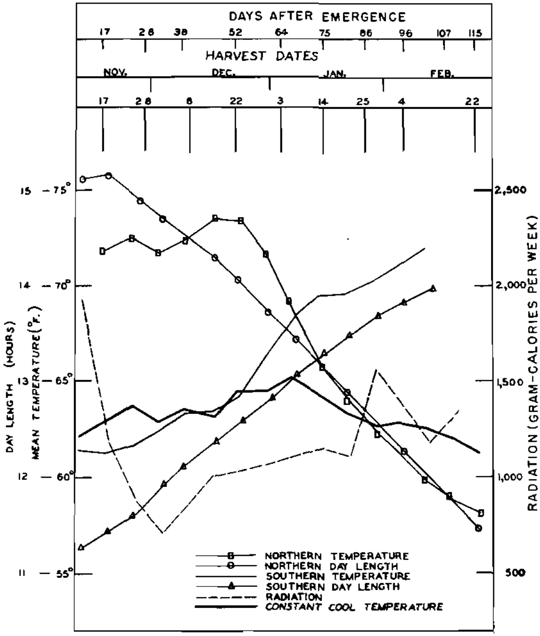


Figure 2.—Environmental conditions in the winter of 1934–35, showing mean temperature, day length, and radiation (measured as gram-calories per square centimeter) for weeks ending at points designated on the various graphs, and days after emergence of plants of the northern and southern series, N34 and S34.

Light-intensity readings were secured at the United States Weather Bureau station about one-fourth of a mile from the greenhouse. Light intensity was greater in 1935–36 than in 1934–35. However, there were 2 weeks of dark days in January 1936 and these caused a noticeable slowing down of tuber growth.

In order to simplify matters as much as possible the first part of the discussion (pp. 765–785) of results will deal with the influence of

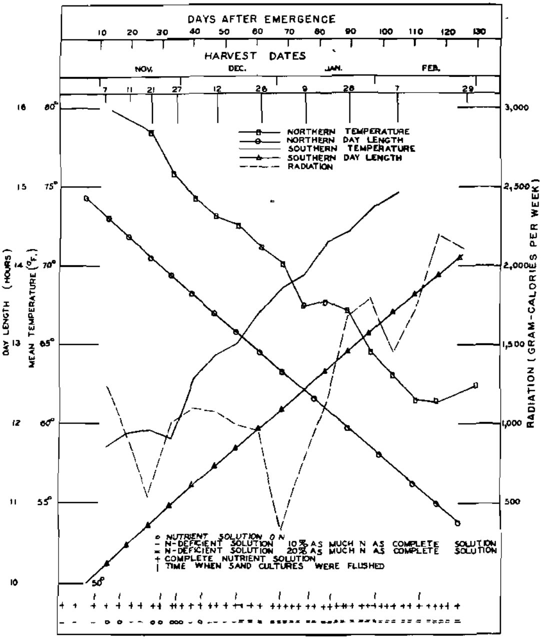


FIGURE 3.—Environmental conditions for 1935–36 treatments, showing mean temperature, day length, and radiation (measured as gram-calories per square centimeter) for weeks ending at points designated on the various graphs, and days after emergence of plants of the northern and southern series, N+, and N−, S+, S−. Symbols at bottom indicate date on which nutrient solutions were applied as explained by legends within the figure.

the environment on one strain (the very early strain No. 12) and the latter part (pp. 785–788) will deal with the differences between strains under the different conditions.

TABLE 1.—*Essential characteristics of the various treatments used in growing strains of Triumph potatoes*

Treatment designation		Day length	Range of mean daily temperature during each treatment	Season when plants were in greenhouse	Nitrogen in nutrient solution	Plants of each strain harvested on each date
Symbol	Descriptive name					
		Hours	*° F.*			*Number*
11C	11-hour, cool	11	62 -65	Oct. 20, 1934, to Feb. 22, 1935	Complete	2
16C	16-hour, cool	16	62 -65	do	do	2
S34	Southern	11 -14	61 -72	Nov. 1, 1934, to Feb. 4, 1935	do	3
N34	Northern	14.5-11.6	72.5-58	Nov. 1, 1934, to Feb. 22, 1935	do	3
S+	Southern, balanced solution.	10 -14.1	58.5-75	Oct. 25, 1935, to Feb. 29, 1936	do	4
S−	Southern, nitrogen-deficient.	10 -14.1	58.5-75	do	10-20 percent.[1]	4
N+	Northern, balanced solution.	14.9-10.8	80 -61	do	Complete	4
N−	Northern, nitrogen-deficient.	14.9-10.8	80 -61	do	10-20 percent.[1]	4
S++	Southern ++N[2] (bright sun).	11.7-15	65 -75	Mar. 2, 1935, to June 1, 1935	++	4
S+−	Southern +−N[2]	11.7-15	65 -75	do	+−	4
S−−	Southern −−N[2]	11.7-15	65 -75	do	−−	4
S−+	Southern −+N[2]	11.7-15	65 -75	do	−+	4

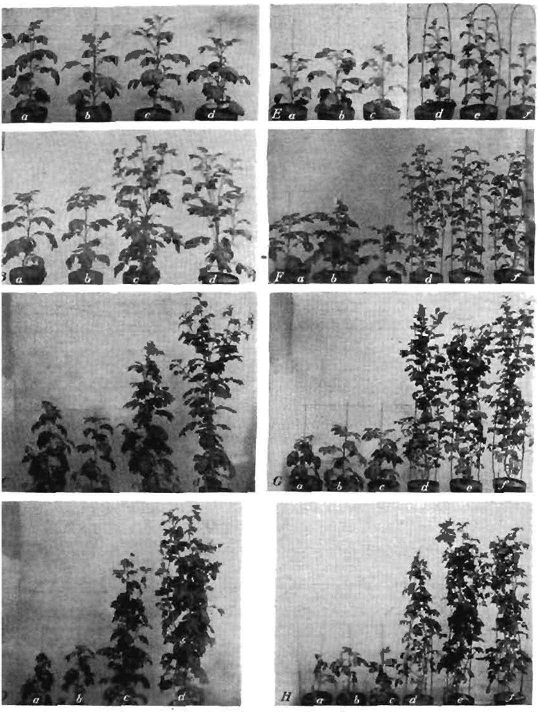
[1] 10-20 percent of the nitrogen in the complete nutrient solution (see fig. 3).
[2] ++N=complete nutrient solution the first 4 and remaining weeks; +−N=complete solution first 4 weeks and after that solution with 10 percent as much nitrogen (or −N); −−N=nitrogen-deficient solution both periods; −+N=nitrogen deficient solution only during the first 4 weeks.

RESPONSE OF VARIOUS PLANT PARTS UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

VEGETATIVE GROWTH

Vegetative growth of the plants, as measured principally by dry weight on successive dates, was very much restricted when the days were short and cool in the early part of the season (11-hour cool days (11C)[7] and under southern conditions (S34, S+, S−; figs. 4, 5, 6). Under these conditions vegetative growth was restricted almost entirely to the main axis, the terminal inflorescence of which aborted while in the primordial stages. During most of the season the leaves were a pale green, becoming glabrous quite early, the outer leaf edges turning downward. The elongation of the main axis was practically accomplished by the seventeenth day. After that the increase in weight resulted from leaf expansion, stem thickening, and stolon production. There was relatively little vegetative growth beyond the thirty-fifth day. After the sixtieth to seventieth days there generally was no increase in vegetative dry weight but frequently a decrease (fig. 5). This decrease was most evident in plants grown with 11-hour cool days (11C), and with those under southern conditions in 1934-35 (S34). It was accompanied by the yellowing and death of lower leaves. These early short-day plants were relatively very leafy as shown by the high leaf/stem ratios (table 2). The early vegetative growth rate and total vegetative growth were greater with 11-hour cool-day plants than with plants grown under southern conditions. There were no consistent differences in the leaf/stem ratios of the two treatments.

[7] Symbols in parentheses refer to treatments outlined in table 1.



:URE 4.—Vegetative growth of potato plants under various combinations of lay length and temperature, 1934–35: *A–D*, Strains 12 (*a*, *c*) and 23 (*b*, *d*) exposed to 11 (*a*, *b*) and to 16 hours of light (*c*, *d*) in treatments 11C and 16C, respectively. Dates and number of days after emergence when photographs were taken: *A*, November 7, 18 days; *B*, December 8, 49 days; *C*, January 3, 75 days; *D*, January 25, 97 days. *E–H*, Strains 12 (*a*, *d*) 22 (*b*, *e*), and 23 (*c*, *f*) grown under southern (*a–c*) and under northern (*d–f*) conditions. Dates and number of days after emergence when photographs were taken: *E*, November 28, 28 days; *F*, December 22, 52 days; *G*, January 14, 75 days; *H*, February 1, 97 days. Height of plants may be gauged by diameter of the 10-inch pots used. Data secured from strain 22, an intermediate-season line, are intermediate between those for strains 12 and 23, but are not published.

Vegetative growth was very extensive when days were long in the rly part of the season as with either 16-hour cool days (16C) or rthern conditions (N34. N+. N−) (figs. 5 and 6). With these

conditions there was very little or no more elongation of the main axis than with short-day plants, but there was very extensive growth of laterals from nodes on all parts of the plants and numerous flower clusters developed (fig. 4). The rapid early growth rate continued till shortly after the sixty-second day after which it decreased slightly. The extent to which these 16-hour cool-day plants were less leafy

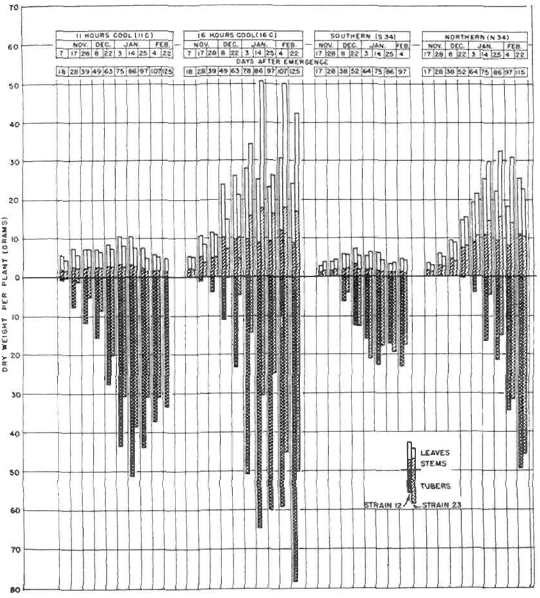


FIGURE 5.—Mean dry weight per plant (in grams) in various parts of plants of two potato strains harvested on successive dates and grown in 1934–35 with 11- and 16-hour cool days (11C and 16C) and under northern and southern conditions (S34 and N34).

than those grown with 11-hour cool or southern days was shown by the very much lower leaf/stem ratios (table 2). These plants matured 15 to 20 days later than the 11-hour-cool or southern-type plants.

In long hot northern days (N34 and N+) the early growth was not exceptionally rapid. Leaf development was proportionately less than with southern-day plants ((S34 and S+), but stem development was greater, as is shown by the dry weights (figs. 5 and 6) and the leaf/stem ratios (table 2).

When a shortage of nitrogen occurred in the nutrient solution vegetative growth was very much inhibited. Under southern conditions the vegetative growth of nitrogen-deficient plants was only about 60 percent of that of plants given a complete nutrient solution (S+ S−, fig. 6). With plants grown with both complete and nitrogen-deficient solutions vegetative growth practically ceased before the

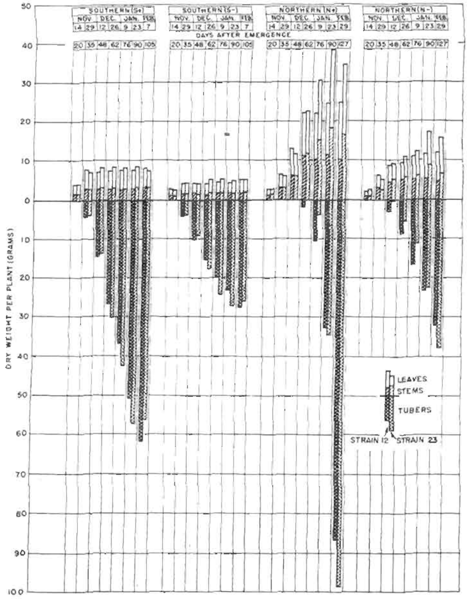


FIGURE 6.—Mean dry weight per plant (in grams) in various parts of plants of two potato strains harvested on successive dates and grown in 1935–36 under southern and northern conditions with a balanced nutrient solution (S+, N+) and with a nitrogen-deficient solution (S−, N−).

thirty-fifth day, the short cool days having been a greater factor in inhibiting vegetative growth than the absence of nitrogen. This nitrogen deficiency did not alter the leaf/stem ratios. Under northern conditions (N−) the maximum vegetative growth of plants raised on a nitrogen-deficient solution was less than half as great as when nitrogen was applied in abundance N+). Under northern conditions vegetative growth of nitrogen-deficient plants continued until the seventy-fifth day or later. Thus the long warm days early in the

ABLE 2.—*Ratios of dry weight of leaves to stems of strains 12 and 23* [1] *of Triumph potatoes as affected by temperature, day length, nitrogen supply, and time of harvest, 1934–35, 1935–36*

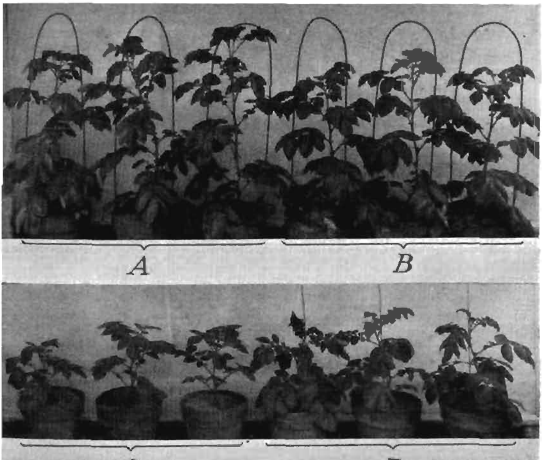
Age of tops when tubers harvested (days)	1934–35								1935–36							
	11-hour cool day (11C) [2]		16-hour cool day (16C) [2]		Southern conditions (S34) [2]		Northern conditions (N34) [2]		Southern conditions				Northern conditions			
									(S+) [2]		(S−) [2]		(N+) [2]		(N−) [2]	
	12	23	12	23	12	23	12	23	12	23	12	23	12	23	12	23
-20	1.78	1.66	1.38	1.50	1.66	1.68	1.24	1.27	1.88	1.83	1.71	1.88	1.45	1.46	1.34	1.39
	1.88	1.47	1.11	1.29	1.46	1.38	1.15	.99	----	----	----	----	----	----	----	----
	----	----	----	----	----	----	----	----	1.98	1.79	1.86	1.87	1.32	1.25	1.11	1.11
-39	1.96	1.49	1.34	1.12	1.29	1.74	1.11	1.13	----	----	----	----	----	----	----	----
-52	1.71	1.16	1.16	1.08	1.35	2.03	1.23	.97	1.83	2.08	1.80	2.10	1.17	1.00	1.09	.90
	1.90	1.94	1.44	1.10	2.18	2.57	1.17	1.05	2.22	2.26	2.11	2.20	1.11	1.00	1.09	.93
-76	2.14	1.87	1.60	1.18	2.75	2.42	1.36	1.07	2.52	2.23	2.27	2.10	1.25	1.05	1.34	1.11
-92	2.28	1.73	1.81	1.38	1.60	2.21	1.38	1.10	2.22	2.11	2.21	2.07	1.27	1.16	1.32	.98
7	2.43	2.75	1.50	1.30	1.38	[3] 3.69	1.29	1.23	----	----	----	----	----	----	----	----
15–107	2.49	2.61	1.50	1.76	----	----	----	----	1.97	1.85	2.20	1.91	----	----	----	----
15 [4]	----	----	----	----	----	----	1.36	1.18	----	----	----	----	----	----	----	----
25–127 [4]	2.18	1.65	1.72	1.52	----	----	----	----	----	----	----	----	1.61	1.18	1.75	1.39

[1] 12=very early strain; 23=very late strain.

[2] Symbols in parentheses are treatment identification. For complete statement concerning these treatments see table 1 and pp. —.

[3] Ratio high because of decay of lower stem portions.

[4] Ratios of weights for last 2 harvest periods not entirely dependable because of loss of portions of dead lower leaves by shattering.



IGURE 7.—Plants from strain 12 photographed on April 12, 1935, after having been grown for 2 weeks with solutions containing different amounts of nitrogen. Plants of various treatments as designated in table 1 are: *A*, S++; *B*, S+−; *C*, S−−; and *D*, S−+.

season were able to bring about vegetative growth in spite of a serious nitrogen deficiency. However, these nitrogen-deficient northern plants were a trifle less leafy than those receiving a complete balanced solution as shown by lower leaf/stem ratios (table 2).

Under southern conditions when sunlight was good (spring of 1935) decreasing the nitrogen supply 4 weeks after plant emergence resulted in a slight decrease in vegetative growth (S++ to S+−, figs. 7 and 8). When the nutritive solution change was in the other direction

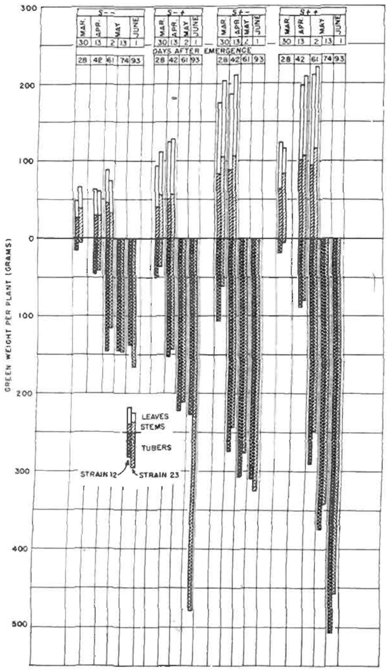


FIGURE 8.—Mean fresh weight per plant (in grams) in various plant parts of plants of two potato strains harvested on successive dates when grown in the spring of 1935 under southern conditions with different amounts of nitrogen in the nutrient solutions. Plants given treatment S− − continually received a nitrogen-deficient solution. At the close of the first 4 weeks the solution given S− + plants was changed from a nitrogen-deficient solution to a balanced solution and S+ − was changed from balanced to a nitrogen-deficient solution. Plants of S+ + received a balanced solution continuously.

there was a prompt and significant increase in growth (S− − to S− +, figs. 7 and 8). Following the shift from nitrogen deficiency to abundance there was little or no increase in stem growth, but all leaves were greatly enlarged and changed from a stiff light green to a more flexible dark green. With the shift from an abundance to a deficiency of nitrogen, plants became more compact as a result of the inhibition of stem elongation and the cessation of leaf differentiation. Apparently there was sufficient nitrogen reserve within the plants to supply them for some time so that the reduction in amount of nitrogen in the nutrient solution did not bring about an acute shortage in 33 days. However, with the plants grown the first 4 weeks with a limited amount of nitrogen the increase greatly accelerated growth, but never to the point where it equaled that of plants of either the S+ + or the S+ − series. Plants of these treatments were not dried at harvest time; hence ratios comparable to those of other series could not be calculated.

STOLON GROWTH

Stolon growth of the plants in the several treatments varied greatly in amount, duration, and type. The inhibition or cessation of stolon growth was inseparably associated with the initiation and development of tubers. Short days early in the season always resulted in very small stolon systems consisting of short primary stolons at each of the underground nodes with tubers terminating these stolons within the first few weeks (figs. 9 to 12). Lateral stolons were few and short, and branch stolons occurred very rarely. All nontuberous stolons or stolons bearing small tubers, especially if they occurred at one of the upper nodes, were rapidly resorbed shortly after the thirtieth day. Increasing the day length or temperature, either gradually or abruptly, did not alter the stolon growth of such plants.

When the days were long early in the season the stolon systems were very extensive because of long stolons and numerous lateral and branch stolons. Under northern conditions with the long warm days early, the stolon growth was more extensive than with 16-hour cool days. This was mostly the result of delayed tuberization. Resorption of nontuberous stolons began sometime after the fiftieth day—according to time of tuberization and continued till the end of the season. However, with less or no dominance of a few early-formed tubers more small tubers developed to appreciable size and fewer tuberous stolons appeared to be resorbed than when the growth of a few tubers became dominant, as with the plants having short days early in the season. This lack of dominance was most apparent with northern-condition plants (figs. 10, 13, and 14).

With a low nitrogen supply stolon growth was restricted only a trifle under southern conditions but to a very pronounced extent under northern conditions (figs. 11 to 14). These differences were again closely associated with tuber initiation. Under northern conditions with a nitrogen deficiency (N−, fig. 14) the stolon growth was quite similar to that under southern conditions with a balanced solution (S+, fig. 11). However, with northern nitrogen-deficient plants stolons were longer than with southern plants receiving a balanced solution, and lateral and branch stolons were quite numerous, whereas they were practically absent from the latter. The nitrogen-deficient northern plants also had a considerable number of nontuberous stolons or stolons with very small tubers until the end of

the season. This condition was almost absent from southern ba anced-solution plants. This finding is in harmony with other dat which have shown northern plants grown with a nitrogen deficienc to be more vegetative than southern plants grown with a balance solution.

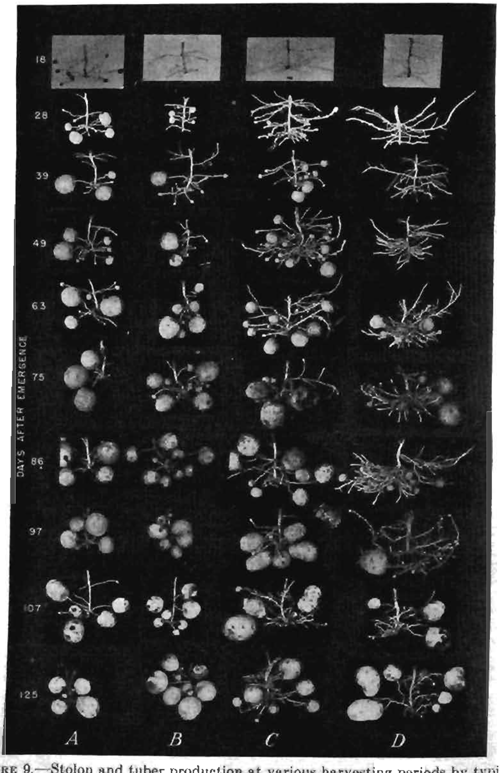


FIGURE 9.—Stolon and tuber production at various harvesting periods by typical plants of two potato strains grown with 11-hour and 16-hour cool days in 1934–35 (11C and 16C). Numbers at left indicate days after emergence: *A*, *B*, Short cool day (11C); *C*, *D*, long cool day (16C); *A* and *C*, strain 12; *B* and *D*, strain 23.

During a period of good sunshine under southern conditions the stolon system of balanced-solution plants differed from nitrogen-deficient plants only in being a trifle longer and in having a few more laterals, all systems being relatively very simple and not altered by nutritional changes made at the close of the first 4 weeks.

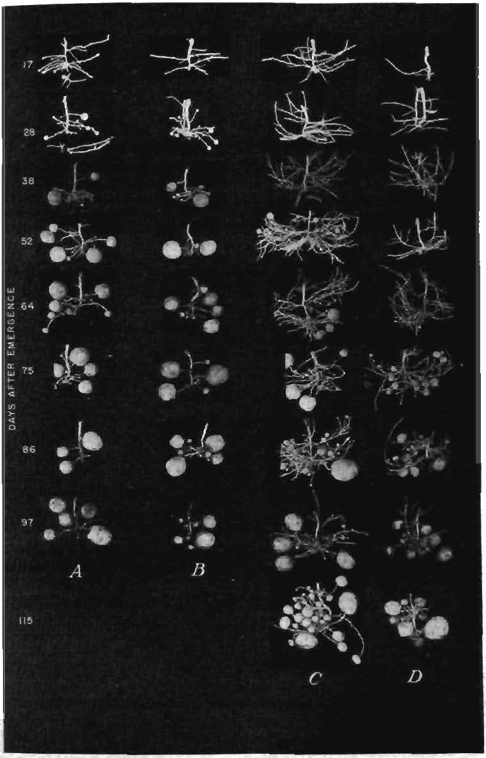


FIGURE 10.—Stolon and tuber production at various harvesting periods, by typical plants of two potato strains grown under southern and northern conditions in 1934–35 (S34 and N34). Numbers at left indicate days after emergence: *A*, *B*, Southern conditions (S34); *C*, *D*, northern conditions (N34); *A* and *C*, strain 12; *B* and *D*, strain 23.

TUBER DEVELOPMENT

The earliest tubers were produced by 11-hour cool-day plants and the latest by northern-day plants. With strain 12 the first tubers were differentiated by the 11-hour cool-day plants about the twelfth day, the southern plants the eighteenth day, the 16-hour cool-day plants a little later, and the northern plants about the forty-eighth day. Nitrogen shortage hastened tuberization at least 2 weeks under northern conditions (figs. 13 and 14) but did not accelerate tuber initiation appreciably under southern conditions (figs. 11 and 12).

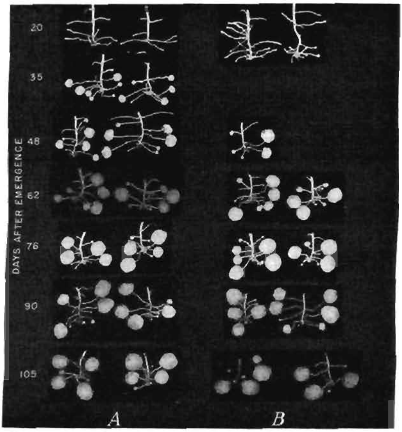


FIGURE 11.—Stolon and tuber production at various harvesting periods by two plants of two potato strains grown under southern conditions in 1935–36 with complete nutrient solution (S+). Numbers at left indicate days after emergence: *A*, Strain 12; *B*, strain 23.

When short, cool days occurred early in the season the first tubers were differentiated from the terminal buds of the primary stolons located at the lower nodes, but at most only a few days elapsed before all primary stolons and all other stolon axes were similarly terminated. With this early tuberization stolon elongation ceased and no new stolon growth occurred. Tuberization was a relatively abrupt process with these short-day plants. The earlier tuberization occurred, the more abruptly did it seem to take place.

When the days in the early part of the season were long, tuberization not only occurred much later than when they were short, but the process was very gradual. With the decreasing day length and declining temperature of the northern conditions tuberization occurred later and again more gradually than with the 16-hour constantly cool days. With these long-day plants the early tuberization generally began at the terminal buds of only a few primary stolons.

Stolon growth reached its peak at about the time of tuberization, even though many stolon buds did not tuberize.

A deficiency of nitrogen in the nutrient solution hastened tuber setting by at least 2 weeks under northern conditions but caused little difference under southern conditions (figs. 12 and 14).

When the days in the early part of the growth period were short, all tubers were set within a comparatively short time early in the season, but when these initial days were long, the process was more attenuated and the maximum number was set later (table 3). The maximum number setting was two to three times greater when early

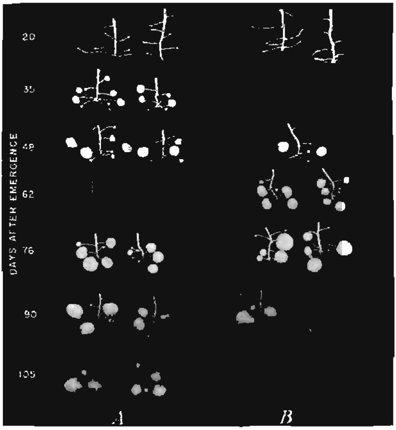


FIGURE 12.—Stolon and tuber production at various harvesting periods by two plants of two potato strains grown under southern conditions in 1935–36 with a nitrogen-deficient solution (S—). Numbers at left indicate days after emergence: *A*, Strain 12; *B*, strain 23.

days were long than when they were short. With both groups the maximum number of tubers occurred at about the same time as did the maximum vegetative weight. With later harvests these maxima in tuber number and vegetative weight both declined. These trends were least defined in the continual cool-day plants. Apparently with the declining plant vigor the smaller tubers were resorbed. The number of tubers weighing more than 1 gm. was also greater with early long- than with early short-day plants.

A deficiency of nitrogen greatly decreased the number of tubers under northern conditions, but under southern conditions the decrease both in total number and in larger tubers was again relatively very much less.

The total yield of tubers and the rate of growth during various periods were very greatly influenced not only by the experimentally altered factors of temperature, day length, and nutrition but also

by the intensity of the natural daylight and by the intensity of l used to extend the natural photoperiod (table 4). The tuber-we increase was relatively high early in the season with the treatm that had short days early in the season. When the early days w warm and long the greatest tuber-weight increase occurred in latter part of the season (table 5).

Plants grown with the 11-hour cool days had relatively the m rapid increase during the first 28 days. After that the daily tu dry-weight production was greater during each succeeding inte till the seventy-sixth day. There was a very slight slowing up of

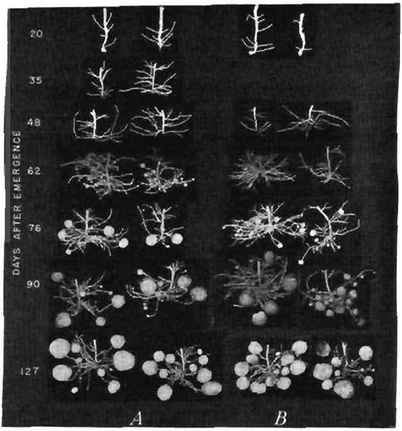


FIGURE 13.—Stolon and tuber production at various harvesting periods, by plants of two potato strains grown under northern conditions in 1935- with a complete nutrient solution (N+). Numbers at left indicate days a emergence: *A*, Strain 12; *B*, strain 23.

tuber increase rate prior to the forty-ninth day when the daylight w the poorest of the season. After the seventy-sixth day tubers co tinued to increase in weight but at a greatly reduced rate. After t ninetieth day there was a decrease in dry weight of tubers.

The tuber growth rates of the southern plants were relatively rap early in the season. During periods of low light intensity the grow rate was relatively low (figs. 2 and 3). The tuber growth rates southern nitrogen-deficient plants were much the same as those plants grown with balanced solutions except that they showed a mo rapid rate of increase early followed by a slower rate of increase (S and S+).

When days were long and cool early in the season (16C) tuberizati was not only later than when days were short and cool (11C) but t very early tuber growth was also a little slower. From the for ninth to the seventy-fifth day the growth rate of 16-hour cool-d

plants continued to increase. After the eighty-sixth day the tubers of 11-hour cool- and southern-day plants had stopped growing or were losing weight. The northern plants with their long warm early days were slowest to tuberize. Consequently their period of most rapid relative increase was always a number of days later than that of plants under any other growing conditions. Their rate of increase was still very rapid till the tests were terminated and the plants were beginning to mature.

The tuberization efficiency of a strain or treatment may be judged by the daily dry weight increase of tubers per gram of dry leaf weight.

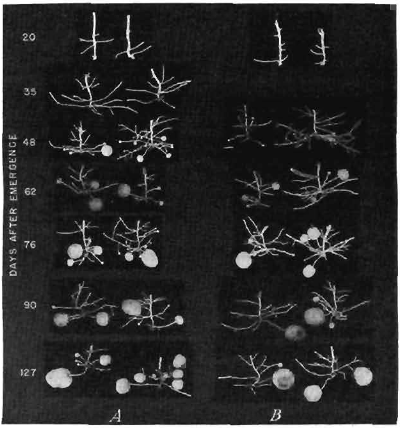


FIGURE 14.—Stolon and tuber production at various harvesting periods, by two plants of two potato strains grown under northern conditions in 1935–36 with a nitrogen-deficient solution (N−). Numbers at left indicate days after emergence: *A*, Strain 12; *B*, strain 23.

On this basis 11-hour cool-day plants were by far the most efficient early in the season (table 5). The southern nitrogen-deficient plants were more efficient during the first 48 days than plants grown with a balanced solution (S−, S+). From then on until the ninetieth day or later the southern plants grown with a balanced solution produced more tuber weight per dry weight of leaves than did the southern nitrogen-deficient plants. With plants under northern conditions tuber production per weight of leaf per day was much lower than for southern plants until very late in the season when the southern plants were maturing more rapidly. Northern nitrogen-deficient plants had a higher rate than balanced-solution plants till after the seventy-sixth day (N+, N−). Then leaves of the nitrogen-deficient plants became yellow or died and were very inefficient during the cool short days at the close of the period, whereas leaves of the balanced-solution plants continued active.

Table 3.—*Mean number of tubers weighing more than 1 gm. and total number per plant, for strains 12 and 23* [1] *of Triumph potatoes, as affected by temperature, day length, nitrogen supply, and time of harvest, 1934–35, 1935–36*

Age of tops when tubers were harvested (days)	1934–35								1935–36							
	11-hour cool day (11C) [2]		16-hour cool day (16C) [2]		Southern conditions (S34) [2]		Northern conditions (N34) [2]		Southern conditions				Northern conditions			
									(S+) [2]		(S−) [2]		(N+) [2]		(N−) [2]	
	12	23	12	23	12	23	12	23	12	23	12	23	12	23	12	23
TUBERS WEIGHING MORE THAN 1 GM. PER PLANT																
17–20	3.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	4.5	4.0	3.5	0	4.5	2.5	0	0	----	----	----	----	----	----	----	----
35	----	----	----	----	----	----	----	----	5.5	5.2	4.0	3.5	0	0	0	0
38–39	4.5	6.5	7.5	0	4.0	3.3	0	0	----	----	----	----	----	----	----	----
48–52	4.5	3.5	17.0	0	8.0	4.0	3.0	0	5.5	4.8	4.0	3.5	0	0	2.0	0
62–64	6.5	12.0	8.5	3.5	6.7	5.7	4.7	0	6.2	5.7	4.5	4.7	4.5	0	2.7	2.2
75–76	9.0	9.5	6.5	4.5	6.0	5.0	9.0	9.0	5.2	5.5	5.0	4.5	4.7	5.0	2.5	2.5
86–90	8.5	8.0	8.0	6.0	3.3	5.0	8.0	6.6	5.7	6.5	3.8	3.6	6.6	8.2	3.0	2.2
97	6.5	5.0	7.0	3.0	8.0	5.0	8.5	5.5	----	----	----	----	----	----	----	----
105–107	6.0	9.5	9.0	3.0	----	----	----	----	5.0	5.0	4.5	3.5	----	----	----	----
115	----	----	----	----	----	----	13.5	8.0	----	----	----	----	----	----	----	----
125–127	6.0	10.0	8.6	5.3	----	----	----	----	----	----	----	----	10.0	11.5	5.2	3.0
TOTAL TUBERS PER PLANT																
17–20	15.0	3.3	0	0	8.0	0	0	0	1.0	0.3	2.7	----	----	----	----	----
28	11.0	7.0	17.5	0	15.5	12.5	----	----	----	----	----	----	----	----	----	----
35	----	----	----	----	----	----	----	----	15.7	15.0	8.2	7.7	----	----	----	----
38–39	12.0	12.5	16.0	2.5	18.3	11.5	.0	0	----	----	----	----	----	----	4.5	0
48–52	16.0	9.0	43.5	----	16.3	15.0	11.7	0	12.7	13.5	7.7	8.5	----	----	14.0	6.5
62–64	11.0	15.5	24.5	19.0	15.0	14.0	34.3	0	12.7	12.2	7.5	8.5	39.0	4.8	13.5	7.0
75–76	15.0	15.0	25.0	39.5	10.6	9.0	37.3	27.0	12.5	8.5	7.2	7.5	17.0	24.8	10.2	8.2
86–90	11.5	14.0	24.0	39.0	5.0	10.0	43.6	28.6	9.2	9.5	6.2	7.0	35.0	53.2	9.0	8.7
97	11.5	5.5	21.5	46.0	9.3	8.0	34.5	19.6	----	----	----	----	----	----	----	----
105–107	11.0	9.5	22.0	10.0	----	----	----	----	9.0	8.0	5.8	6.5	----	----	----	----
115	----	----	----	----	----	----	22.0	16.5	----	----	----	----	----	----	----	----
125–127	6.0	11.0	11.3	9.6	----	----	----	----	----	----	----	----	26.0	37.0	9.2	5.0

[1] 12=Very early strain; 23=very late strain.
[2] Symbols in parentheses are treatment identification. For complete statement concerning these treatments see table 1 and pp. 761–763.

Table 4.—*Mean fresh weight of tubers as grams per plant for strains 12 and 23* [1] *of Triumph potatoes as affected by temperature, day length, nitrogen supply, and time of harvest, 1934–35, 1935–36*

Age of tops when tubers were harvested (days)	1934–35								1935–36							
	11-hour cool day (11C) [2]		16-hour cool day (16C) [2]		Southern conditions (S34) [2]		Northern conditions (N34) [2]		Southern conditions				Northern conditions			
									(S+) [2]		(S−) [2]		(N+) [2]		(N−) [2]	
	12	23	12	23	12	23	12	23	12	23	12	23	12	23	12	23
17–20	6.8	0.1	0	0	0.6	0	0	0.07	0.07	0.03	0.13	0	0	0	0	0
28	64	10	9	0	7	3	0	.0	----	----	----	----	----	----	----	----
35	----	----	----	----	----	----	----	----	35	30	34	31	0	0	.6	0
38–39	96	44	31	.4	52	34	0	.0	----	----	----	----	----	----	----	----
48–52	117	67	91	.8	101	96	5.3	.0	107	107	68	65	0	0	26	2.5
62–64	193	159	181	41	114	145	36	.4	177	192	103	114	16	.2	68	45
75–76	343	219	332	115	149	116	133	38	232	256	130	153	108	31	116	85
86–92	306	234	439	212	108	123	156	109	300	328	149	171	217	250	157	148
97	274	186	378	311	151	105	206	187	----	----	----	----	----	----	----	----
105–107	229	214	360	266	----	----	----	----	363	329	187	180	----	----	----	----
115	----	----	----	----	----	----	299	248	----	----	----	----	----	----	----	----
125–127	210	----	455	298	----	----	----	----	----	----	----	----	489	560	209	220

[1] 12=Very early strain; 23 =very late strain.
[2] Symbols in parentheses are treatment identification. For complete statement concerning these treatments see table 1 and pp. 761–763.

TABLE 5.—*Increase or decrease in dry weight of tubers, expressed as the mean daily amount in milligrams per plant and per gram of dry leaf, strains 12 and 23* [1] *of Triumph potatoes, as affected by temperature, day length, nitrogen supply, and time of harvest, 1934–35, 1935–36*

PER PLANT BASIS

Age of tops when tubers were harvested (days)	11-hour cool day (11C) [2]		16-hour cool day (16C) [2]		Southern conditions, 1934–35 (S34) [2]		Northern conditions, 1934–35 (N34) [2]		Southern conditions, 1935–36 (S+) [2]		Southern conditions, 1935–36 (S−) [2]		Northern conditions, 1935–36 (N+) [2]		Northern conditions, 1935–36 (N−) [2]	
	12	23	12	23	12	23	12	23	12	23	12	23	12	23	12	23
	Milli-grams	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*
17–28	45	1	0	0	3	0	0	0	1	3	2	0	0	0	0	0
28	706	107	106	0	42	33	0	0								
35									285	253	384	262	0	0	4	0
38–39	358	356	257	3	599	378	0	0								
48–52	405	348	715	2	441	620	38	0	783	778	454	399	0	0	225	204
62–64	842	821	889	330	288	714	298	1	885	1,160	367	434	108	0.4	420	377
75–76	1,325	874	2,295	807	605	344	1,155	433	725	878	330	475	629	257	551	417
86–90	1,000	719	1,266	1,473	492	167	459	962	990	1,063	224	208	1,600	2,216	480	789
97	−668	−688	−446	−507	518	167	1,185	1,505								
105–107	−677	4	−49	2,024					735	−63						
115							826	785								
125–127			1,060	2,761							301	94	1,390	1,680	244	420

DRY-LEAF-WEIGHT BASIS

Age of tops when tubers were harvested (days)	11C 12	11C 23	16C 12	16C 23	S34 12	S34 23	N34 12	N34 23	S+ 12	S+ 23	S− 12	S− 23	N+ 12	N+ 23	N− 12	N− 23
17–20	25	0.5	0	0.0	3	0	0	0.0	0.1	1	1	0	0	0	0	0
28	169	35	24	0	21	13	0	0								
35									22	19	35	32	0	0	1	0
38–39	75	92	42	.6	200	124	0	0								
48–52	87	84	73	.3	105	175	6	0	33	38	45	39	0	0	16	2
62–64	168	187	63	35	70	170	34	.1	47	49	36	38	4	0	24	22
75–76	209	175	141	55	115	90	94	33	35	39	31	37	14	5	26	19
86–90	14	144	76	69	153	61	34	60	46	47	20	17	32	32	20	27
97	−106	−164	−30	−17	200	60	104	89								
105–107	−143	0	−3	62					35	−3	20	−8				
115							67	54								
125–127			63	10									19	25	7	12

[1] 12=Very early strain; 23=very late strain.
[2] Symbols in parentheses are treatment identification. For complete statement concerning these treatments see table 1 and pp. 771–763.

On the basis of dry weight of tubers produced for each gram of lea and stem dry weight, i. e., tuber/top ratios, the most efficient plant very early in the season were the 11-hour cool-day plants (11C), fol lowed rather closely by the southern plants (S34, S+, S— (table 6)) The ratios with these plants, which it will be remembered had rela tively small vegetative growth, remained high all season. The ratio of 16-hour cool-day plants were much lower than those of the 11-hou cool-day plants, but those of northern plants were generally still lowe Early in the season the tuber/top ratios of nitrogen-deficient plant were always higher than those of the corresponding balanced-solutio plants, but late in the season they were lower. As vegetative growt increased the tuber/top ratio decreased. The conditions that wer most favorable for maximum vegetative growth were least favorabl for most efficient tuber production when calculations were made on th basis of a unit of leaf area.

TABLE 6.—*Ratios of dry weight of tubers to that of vegetative parts (tops) for strain 12 and 23*[1] *of Triumph potatoes as affected by temperature, day length, nitroge supply, and time of harvest, 1934–35, 1935–36*

Age of tops when tubers were harvested (days)	1934–35								1935–36							
	11 hour cool day (11C)[2]		16 hour cool day (16C)[2]		Southern conditions (S34)[2]		Northern conditions (N34)[2]		Southern conditions				Northern conditions			
									(S+)[2]		(S−)[2]		(N+)[2]		(N−)[2]	
	12	23	12	23	12	23	12	23	12	23	12	23	12	23	12	23
17–20	0.14		0.00	0.00	0.17	0.00	0.00	0.00		0.01	0.01	0.00	0.00	0.00	0.00	0.00
28	1.07	0.20	.10		.14	.08	.00	.00								
35									0.57	.50	1.01	.93	.00	.00	.11	.00
38–39	1.64	.72	.29	.03	1.13	.73	.00	.00								
48–52	2.23	1.32	.46	.04	1.75	2.46	.03	.00	2.07	1.60	2.34	2.22	.00	.00	.35	.03
62–64	3.28	2.75	.90	.22	3.06	3.35	.22	.08	3.46	3.41	3.80	3.48	.08	.00	.94	.52
75–76	4.16	3.84	1.84	.42	3.75	4.08	.67	.16	4.57	4.98	4.46	4.64	.49	.12	1.52	.92
86–92	4.87	5.11	2.58	.61	5.47	5.62	1.00	.48	6.36	6.40	5.35	5.49	1.34	.85	2.05	1.29
97	5.86	6.25	2.59	.42	5.06	4.38	1.94	1.05								
105–107	6.33	5.92	1.95	.91					7.28	7.26	5.24	5.04				
115							1.96	2.06								
125–127	6.85	5.61	3.27	1.18									3.72	3.03	2.70	2.42

[1] 12=Very early strain; 23=very late strain.
[2] Symbols in parentheses are treatment identification. For complete statement concerning these treatments see table 1 and pp. 761–763.

It is of interest to note the comparative merits of the various treatments for total tuber production during given periods of time in various parts of the growing season. Plants that started with cool short days and tuberized early produced a large weight of tubers per plant per day during a 50- to 60-day period, with heavy production under way relatively early (11C, 16C, southern, table 5). When tuberization was once initiated long warm days did not prevent great tuber increase even though they might have prevented or delayed initiation of tubers. With northern plants the maximum tuber production per day was approximately twice as great as the maximum for southern plants that were growing at the same time but this rate was not attained until the seventy-sixth day or later. Prior to the sixty-second or seventy-sixth day the nitrogen-deficient plants under northern conditions (N—) were yielding more per day than the balanced-solution plants. Under southern conditions the

tuber production of nitrogen-deficient plants (S−) was higher than that of balanced-solution plants (S+) till the thirty-fifth day. After that the S− plants produced much less per day than the S+ plants as the leaves were chlorotic and were approaching or had reached senility.

The final yield of tubers was always greater with the plants that had the larger tops. With such large plants production per leaf unit was relatively low (table 5), but the total leaf system was so great that the total tuber production was relatively great. At any time up to about 75 to 90 days after emergence the southern plants were more productive than the northern plants. After that time tuber yields of southern plants increased very little, but from then on half of the tuber weight of northern plants was produced. The production of poorly illuminated southern plants was relatively low, whereas that of well-illuminated plants was finally equal to or greater than that from 11-hour cool-day plants. The 16-hour cool-day plants attained higher early tuber production rates per gram of leaf weight than northern plants but the final production rate was greater with the latter.

A nitrogen deficiency curtailed total tuber production more under northern than under southern conditions. The nitrogen-deficient plants were not able to benefit by the cool short days at the close of the northern season because of the great reduction in leaf area,the reduced amount of chlorophyll, and the early death of the plants. In an experiment conducted in the spring of 1935, when a nitrogen deficiency existed all season under southern conditions final tuber production was about one-third as great as when nitrogen was supplied in adequate amounts all season (S− − and S++, fig. 8). Increasing the nitrogen supply from the nitrogen—deficient solution to a complete solution (S−+) at the end of 2 weeks brought about a great increase in yield. Decreasing the amount of nitrogen at the end of 4 weeks (from the complete solution to the nitrogen-deficient solution) resulted in about a 30-percent reduction in tuber yield (S+− as compared with S++). However, this yield was still greater than when nitrogen was deficient only early in the season (except with strain 23). Stated differently, this means that with the early strain under southern conditions a high nitrogen supply for 4 weeks early in the season was worth considerably more for tuber production than a high nitrogen supply during the last 8 or 9 weeks, whereas the late strain was able to survive this early deficit and respond to the later abundantly supplied nitrogen. In fact the late strain (23) yielded slightly more with the S−+ solution than with the S++ solution.

Under southern conditions tubers were more uniform, larger, and of better type than under northern conditions (figs. 10 to 14). Under northern conditions a few tubers may have been larger but there was more variation in tuber size than with southern plants. With continually cool days tubers were larger than with other treatments—the largest and most elongated having been produced by 16-hour cool days (fig. 9). When nitrogen was deficient the size of southern tubers was less than when nitrogen was abundant, but the range in size was about the same (figs. 11 and 12). Under northern conditions with a nitrogen deficiency one or two tubers generally grew to great size while the others remained relatively small (fig. 14).

DRY MATTER CONTENT OF VARIOUS PLANT PARTS

The percentage of dry matter in the leaves of both the 11-hour and the 16-hour cool-day plants was relatively high at the time of the first harvest (table 7). After that the percentage of dry matter decreased till about the thirty-ninth day, after which it increased gradually with a very rapid increase as the plants became senile. With southern and northern plants the initial high percentage was less apparent. During all the time prior to the period of senility the percentage in the leaves of 16-hour plants was higher than in those of 11-hour plants. During the first 60 days the dry-matter percentage was greater in the leaves of northern than in those of southern plants.

TABLE 7.—*Percentage of dry matter in leaves, stems, and tubers of strains 12 and 23* [1] *of Triumph potatoes, as affected by temperature, day length, nitrogen supply and time of harvest, 1934–35, 1935–36*

Age of tops when tubers were harvested (days)	11-hour cool day (11C) [2]		16-hour cool day (16C) [2]		Southern conditions, 1934–35 (S34) [2]		Northern conditions, 1934–35 (N34) [2]		Southern conditions, 1935–36: Complete nutrient solution (S+) [2]		Southern conditions, 1935–36: Nitrogen-deficient solution (S−) [2]		Northern conditions, 1935–36: Complete nutrient solution (N+) [2]		Northern conditions, 1935–36: Nitrogen-deficient solution (N−) [2]	
	12	23	12	23	12	23	12	23	12	23	12	23	12	23	12	23
DRY MATTER IN LEAVES																
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
17–20	9.8	9.6	10.1	9.7	7.2	7.3	8.0	7.5	8.6	8.7	8.3	7.6	8.9	8.6	8.1	7.[illegible]
28	7.5	7.6	9.3	8.2	7.2	7.2	7.3	6.9								
35									8.1	8.3	7.1	7.2	9.0	8.7	8.1	7.[illegible]
38–39	7.4	7.2	8.0	8.1	8.0	8.3	8.4	9.6								
48–52	7.8	7.8	8.8	8.9	7.9	7.7	9.0	8.0	6.6	7.8	6.7	7.1	9.2	9.2	8.8	8.[illegible]
62–64	7.7	7.2	8.8	8.7	9.8	9.5	10.9	10.4	7.2	7.5	6.2	6.3	9.6	8.6	7.7	8.0
75–76	8.5	7.7	10.4	9.7	12.4	10.2	12.0	10.0	8.4	7.2	6.3	6.1	11.8	10.3	8.8	9.0
86–90	10.9	9.7	10.3	9.9	13.6	11.2	13.3	11.3	9.6	9.7	7.1	6.7	12.4	11.6	9.9	10.0
97	13.9	11.3	11.9	12.6	33.9	38.1	16.0	12.1								
105–107	23.6		19.9	19.2					39.7	37.2	22.7	21.8				
115							29.4	17.9								
125–127	81.4	81.6	73.9	48.6									17.0	32.7	22.5	13.4
DRY MATTER IN STEMS																
17–20	4.5	5.1	4.6	4.6	4.9	4.1	3.9	3.6	3.8	3.9	3.9	3.6	3.6	3.6	3.7	3.[illegible]
28	4.5	4.0	5.2	4.0	3.9	4.0	3.8	3.7								
35			.0	.0					3.8	3.9	4.0	3.9	3.9	4.1	4.7	4.4
38–39	4.0	5.3	4.5	4.1	4.2	4.8	4.2	4.4	3.9	3.9	4.0	3.7	4.2	4.2	5.1	5.5
48–52	4.2	4.4	4.8	4.7	6.5	4.4	4.7	4.6								
62–64	4.2	4.3	4.8	4.9	4.7	4.3	4.9	5.0	3.8	3.9	4.0	3.8	4.8	4.5	5.4	5.7
75–76	4.5	4.6	5.1	5.2	4.2	4.9	4.9	5.0	3.7	3.7	4.0	4.1	4.9	5.1	5.4	6.0
86–90	4.5	4.3	5.2	5.2	6.5	4.9	5.1	6.1	3.9	4.2	4.3	4.6	5.1	5.6	5.8	7.2
97	4.8	4.6	5.4	6.2	5.9	5.9	5.7	5.4								
105–107	5.1	17.5	6.4	6.1					5.2	10.8	10.9	7.8				
115				.0			7.4	6.5								
125–127	26.3	59.4	19.0	9.1									5.5	10.4	6.9	8.2
DRY MATTER IN ALL TUBERS																
17–20	11.8	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	12.3	11.6	11.8	.0	7.9	10.9	.0	.0								
35									12.4	12.6	12.5	12.9	.0	.0	11.6	.0
38–39	12.3	11.7	12.4	10.0	12.5	12.4										
48–52	13.6	12.8	12.1	7.3	12.6	13.4	10.0	.0	13.6	13.0	15.0	14.1	.0	.0	11.6	10.9
62–64	14.3	12.7	13.0	11.4	14.1	14.8	11.6	4.6	15.2	15.7	14.9	15.5	11.9	3.6	13.0	12.4
75–76	16.3	13.9	15.4	12.5	15.3	15.2	12.7	12.6	16.0	16.6	15.3	15.9	12.9	11.6	14.3	13.4
86–90	16.8	16.5	14.8	14.4	16.2	15.8	14.1	14.2	16.9	17.5	15.5	15.9	15.3	13.9	14.8	15.2
97	16.0	16.7	15.9	16.1	15.3	16.8	16.9	17.1								
105–107	16.2	14.5	16.5	17.0					17.0	17.1	14.8	14.4				
115							16.6	18.5								
125–127	15.9		17.3	16.8									18.8	18.6	15.5	17.2

[1] 12=very early strain; 23=very late strain.

[2] Symbols in parentheses are treatment identification. For complete statement concerning these treatments see table 1 and pp. 761–763.

The percentage of dry matter in the stems tended to increase as the season advanced. The percentage in the 16-hour cool-day plants was generally higher than in the 11-hour plants and in the northern than in the southern plants.

With all treatments the percentage of dry matter in the total tuber crop increased rather steadily as the season advanced, but frequently decreased at the time of the last harvest (table 8). This increasing percentage of dry matter was associated with constantly increasing tuber size—the larger tubers consistently having the higher dry-matter percentages (table 8).

TABLE 8.—*Percentage of dry matter in tubers of strains 12 and 23*[1] *of Triumph potatoes, as affected by tuber weight, temperature, day length, nitrogen supply, and time of harvest, 1935–36*

Tuber weight range at harvest and period elapsed since emergence (days)	Southern conditions, 1935–36				Northern conditions, 1935–36			
	Complete nutrient *solution* (S+)[2]		Nitrogen-deficient *solution* (S−)[2]		Complete nutrient *solution* (N+)[2]		Nitrogen-deficient *solution* (N−)[2]	
	12	23	12	23	12	23	12	23
0.01 to 1 gm.:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
20	19.23	16.53	21.15					
35	12.03	11.85	13.30	12.78		11.56		
48	6.86	7.54	9.52	7.58		8.92		10.86
62	8.57	6.88	10.09	11.91	11.90		7.20	7.08
76			11.05	6.73	7.28	11.81	3.51	5.58
90	8.21	8.24	6.57	9.87	7.02	5.81	4.21	4.32
105	10.00	7.10	14.38	3.89				
127					7.76	5.00	7.65	5.00
1.01 to 25 gm.:								
35	12.36	12.61	12.50	12.87				
48	13.60	12.89	14.21	14.40			11.80	
62	12.94	19.89	14.65	15.67	11.82		13.03	12.18
76	13.65	15.34	14.68	16.55	12.29	11.61	13.20	11.89
90	13.82	14.04	14.77	11.44	14.62	13.41	13.02	11.49
105	14.59	16.60	14.60	14.04				
127					16.65	14.48	10.70	7.78(?)
25.01 to 75 gm.:								
48	13.68	13.17	15.43	13.82			11.90	
62	15.33	15.27	15.06	15.44			13.07	12.64
76	16.13	17.76	15.43	16.20	13.33	11.40	14.92	13.48
90	16.57	17.94	15.67	15.44	16.30	14.28	14.13	14.28
105	16.60		14.70	14.23				
127					18.39	18.60	15.75	
75.01 to 150 gm.:								
62	16.10	16.04						
76	15.97	16.44		15.39			14.59	13.97
90	17.40	17.35	15.43	16.54	15.40	14.17	15.52	15.89
105	17.38	17.19	15.07	14.79				
127					19.25	20.65	0	16.45
150.01 gm. or more:								
90								14.44
105								
127					20.09	19.01	16.54	17.85

[1] 12=Very early strain; 23=very late strain.
[2] *Symbols in parentheses are treatment identification. For complete statement concerning these treatments* see table 1 and pp. 761–763.

On all except the latest harvest dates the tubers of the plants that set the earliest tubers had the highest percentages of dry matter. Those from 11-hour cool days and southern conditions had the high percentages, those from 16-hour cool days were intermediate, and those from northern plants were lowest in dry-matter percentage. The early-harvested tubers from nitrogen-deficient plants had higher percentages than tubers from balanced-solution plants but late in the season they had lower. At the last harvest time the northern 16-hour cool-day tubers and tubers from northern high-nitrogen

plants had the highest percentages. These differences are the result of the great differences on any date in the size and physiological age of the tubers produced under various conditions. The tubers of the early short-day plants were always more mature and uniformly larger than those of early long-day plants just as tubers of nitrogen-deficient plants were more mature than those from balanced-solution plants especially under northern conditions. When tubers of about the same physiological age are compared the differences are found to have been very slight.

With 11-hour cool-day and southern plant tubers the percentage of dry matter increased very slowly after midseason and sometimes it decreased slightly. Probably some of the dry matter was being dissipated from the tubers by their own respiration at the high temperatures prevailing late in the life of southern-day plants or in the life processes of the senile plants as they lingered on. The high dry matter in northern tubers late in the season was probably the result of the absence of practically all demands for materials for growth and the greatly reduced rate of respiration of plants and tubers. As a result of all of these conditions there was a great increase in surplus carbohydrates, and general rapid tuber enlargement resulted. The lower percentage of dry matter in the late nitrogen-deficient tubers is attributed to the weak condition of the plants during a prolonged period.

STARCH CONTENT OF TUBERS

Analysis of tubers of different size selected from various treatments on several dates in 1935–36 showed a rather definite tendency for starch content to increase as tuber size increased (table 9). When com-

TABLE 9.—*Percentage of starch (fresh basis) in tubers of strains 12 and 23*[1] *of Triumph potatoes as affected by tuber weight, temperature, day length, nitrogen supply, and time of harvest, 1935–36*

Period elapsed since emergence and tuber weight range at harvest (grams)	Southern				Northern			
	Complete nutrient solution (S+)[2]		Nitrogen-deficient solution (S−)[2]		Complete nutrient solution (N+)[2]		Nitrogen-deficient solution (N−)[2]	
	12	23	12	23	12	23	12	23
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
48 days:—1.01–25	6.60							
62 days:								
1.01–25			9.27	11.09			7.56	
25.01–75	9.29						7.53	
75.01–150	10.74							
76 days:								
1.01–25			10.28				7.91	
25.01–75	10.54		11.03					
75.01–150	10.56						10.12	
90 days:								
25.01–75	12.40	11.59	11.27	10.68	11.34	9.40	9.88	
75.01–150	11.51	11.71	10.71	11.68	9.47	9.13		10.69
150+								9.56
105 days:								
1.01–25	9.00							
25.01–75	11.37		9.87	9.85				
75.01–150	11.74		10.46	10.12				
150+								
127 days:								
1.01–25					10.20		6.92	
25.01–75					11.47		11.24	
75.01–150					13.20	13.96		11.04
150+					14.55	13.43	11.43	12.37

[1] 12=Very early strain; 23=very late strain.

[2] Symbols in parentheses are treatment identification. For complete statement concerning these treatments see table 1 and pp. 761–763.

parisons are made with data from tubers of the same size group, harvested on the same early dates, those from southern plants appear to have had more starch than those from northern plants, but, conversely, on late dates the northern tubers had the higher percentage. However, when tubers of the same physiological age are compared these differences are not very great. It appears that tubers of the same size and same physiological age, or harvested from plants of the same physiological age, have approximately the same percentage of starch and dry matter.

COMPARISON OF RESPONSE OF TWO STRAINS

When conditions were very favorable for extensive vegetative growth, as northern (N34, N+) or with 16-hour cool-days (16C), plants of strain 12 (the first early strain) were frequently largest early in the season but genetic limitations came into play and inhibited the growth of this strain rather abruptly during the early midseason period, bringing on the early death of the plants. With strain 23 (the very late strain) these factors came into play later and more slowly so that the vegetative growth of 23 was greater. Under conditions restricting vegetative growth (southern and 11-hour cool) top growth of strain 23 was only slightly greater or was less than that of strain 12. With a nitrogen deficiency, plants of strain 23 were slightly larger than those of strain 12, responding about as with a complete nutrient solution (figs. 4, 5 and 6).

Plants of strain 12 were always more compact, having noticeably shorter internodes, larger leaves, and leaflets and more and larger folioles (fig. 4). These differences recorded as leaf/stem ratios were very pronounced when the size of the plants of strain 23 exceeded that of strain 12 as with northern or cool 16-hour days (table 2). With southern conditions or nitrogen-deficient nutrition the difference in leafiness was less apparent.

Thus it appears that when conditions were unfavorable for vegetative growth or differentiation early in the season, there was little difference in the growth of the two strains. When conditions in the early part of the season were very favorable for vegetative growth the plants of the late strain grew to be very large, owing to prolongation of growth and differentiation and not to more rapid early growth.

Under all conditions the stolon growth got under way somewhat quicker with strain 12 and was more extensive with this strain until tuberization set in (figs. 9 to 14). After that stolon growth was most extensive on strain 23 until it too set tubers. Whenever stolon growth was very extensive as under northern (N34, N+) or 16-hour cool days (16C) the development of stolons of strain 23 lagged rather far behind that of strain 12, but when early days were short (11C, S+, S34) or plants were grown with a nitrogen deficiency (S− or N−) there was very little difference (figs. 9 to 14).

Tuberization always occurred earlier with strain 12 than with strain 23. With 11-hour cool or southern days it occurred very early with little difference between the strains. When tuberization occurred late, as with northern days, the difference between strains was about 16 days, and with 16-hour cool days it was about 25 days When a nitrogen deficit existed there was little difference between strains in time of tuber setting.

Strain 12 generally set most tubers per plant with southern or 11-hour cool days (table 3). Under northern conditions and 16-hour cool days strain 12 had most tubers early in the season and strain 23 had most in the latter part (except 1934–35 northern). Whenever the number of tubers of 23 exceeded those of 12 it was because of

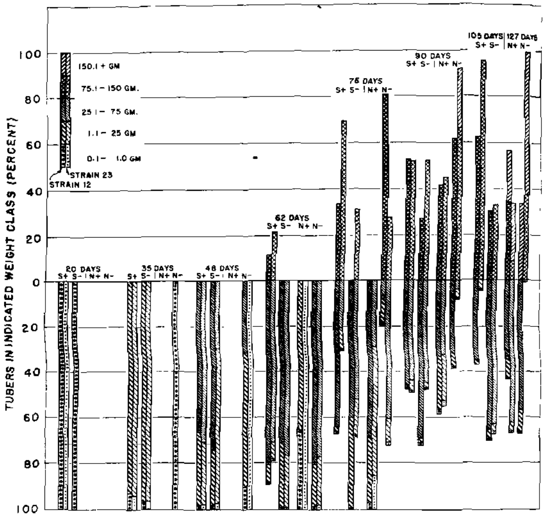


FIGURE 15.—Percentage of total fresh weight of tubers in various tuber-weight classes when harvested the indicated number of days after plant emergence, 1935–36. Letters S and N indicate northern or southern conditions; + and – signs indicate when nutrient solution was balanced or deficient in nitrogen. See table 1 for further information concerning treatments. The two classes of largest tubers are shown in percentage above the zero line, the other classes below.

many tubers having been set late in the season. With a nitrogen deficit there was little difference between strains in number of tubers under southern conditions, but under northern conditions 23 always had fewer.

With both strains many small tubers were resorbed after a peak in numbers had been attained. This peak occurred earlier with 12 than with 23, the difference in the time of peak occurrence corresponding approximately with the difference in time of tuber initiation. The number of tubers weighing in excess of 1 gm. was generally greater with 12 than with 23, although late in the northern season 23 had more in 1935–36.

In the 1935–36 series a higher percentage of the final total weight

' strain 23 occurred in the large-tuber groups than in strain 12 ig. 15). Most large tubers of strain 12 were nearly of the same ze, and none grew to be very large, whereas in strain 23 one or two ıbers were much larger than the others. With strain 12 all tubers ere set within a very short time; with strain 23 tuberization was a ifle more gradual, and slightly fewer were set.

The total production of tuber dry weight of strain 12 exceeded ıat of strain 23 at all early harvest periods with all treatments (fig. and table 4) and weights of tubers continued to be highest with rain 12 in the lots that were constantly cool (11C, 16C). Under ›rthern conditions they continued to be greatest until very late in ıe season. Under southern conditions yields after midseason were ost frequently greatest with strain 23 with either type of nutrition, ıt they were too variable to permit any positive deduction. The ost striking difference was in the ability of plants of strain 23 to ·entually respond much more than those of strain 12 to a liberal trogen supply after the 30-day initial starvation period, as shown treatment S−+ (fig. 8).

Plants of strain 12 were somewhat more efficient in tuber produc- on than those of 23, having very generally had higher tuber/top .tios (table 6). Under southern conditions there was less difference these ratios than under northern or continually cool conditions. he relative lack of adaptation of strain 23 to 16-hour cool-day con- tions is brought out by the extremely low tuber/top ratio of strain ;. Again it is evident that when conditions are adverse for extensive ›getative growth early in the season or all the season, environmental ›nditions almost obliterate genetic differences in tuberization habits.

The mean daily tuber production is a means of measuring tuber- ation capacity at various periods. Under all conditions the daily .te was greater early in the season with strain 12 than with strain 23 able 5). With strain 12 the maximum production rate was attained .rlier in the season and more quickly after tuberization set in than ith strain 23.

On the basis of milligrams of dry tuber weight per gram of dry af weight per day the rates were always highest for strain 12 very ırly in the season (table 5). They continued to be highest with rain 12 under northern conditions till very late in the season. Under ›uthern conditions they were highest with strain 23 in the midseason ›riod.

After analyzing the data in various ways it appears that under ›uthern conditions strain 23 was most frequently capable of pro- ıcing a slightly greater weight of tubers in midseason and later able 5). This was probably due to the ability of the plants to ›main green a little longer than those of strain 12.

On any one date the percentage of dry matter in tubers was gen- ·ally higher for strain 12 than for strain 23, but this was due to fferences in physiological age and size of tubers (tables 7 and 8). n any one date differences were least when tubers of both strains ere nearly of the same size and age as under southern conditions or ith a nitrogen deficiency. With tubers of the same weight groups ıe dry matter percentage was sometimes highest with strain 23, ·obably because of the tendency for the tubers within the group to ; larger than with strain 12.

There was little difference in starch content of the tubers from tl two strains and any differences observed occurred in the same mann as the dry-matter differences (table 9).

SUMMARY AND CONCLUSIONS

PLANT RESPONSE

A. Triumph potato plants grown in 11-hour cool days produc very small plants, with very high leaf/stem and tuber/top ratio These plants set tubers very early on a few short stolons. The dai increase in tuber weight per plant or per gram of dry weight of leav was very great in the early part of the season, but there was no i crease after about the seventy-fifth day. The tubers had a relativel high dry-matter content.

B. Plants grown in cool 16-hour days differed from those grown i cool 11-hour days as follows:

(1) Plants were much larger and continued to grow several weel longer.

(2) Plants were less leafy as shown by lower leaf/stem ratios.

(3) Tuber production occurred several weeks later; tubers we more numerous and were produced on longer stolons.

(4) Total tuber weight was about the same by the time maximu weight was attained by 11-hour plants, after which tuber productio continued to increase with 16-hour plants till finally t was approx mately twice as great.

(5) The tuber/top ratios were always much lower.

(6) The daily increase in tuber dry weight per weight of leaves wa much lower till after midseason.

(7) Dry-matter content of vegetative parts was usually greate dry-matter content of tubers from plants of the same age was abou the same.

C. Plants were produced under "southern" conditions, that i days increased gradually in length and temperature from short co days early to long warm days late in the season, and under "northern conditions with long warm days early and cool short days late. Unde southern conditions the response of the plants was very similar to tha of 11-hour cool-day plants, whereas under northern conditions th response was similar to that of 16-hour cool-day plants.

D. The southern plants differed from the 11-hour plants in th following respects:

(1) Plants were smaller.

(2) Tuberization occurred a trifle later.

(3) The number of tubers was a trifle smaller.

(4) Tuber weight was considerably lower.

(5) The tuber/top ratios were slightly lower.

E. The northern plants differed from the 16-hour cool-day plant in having:

(1) Less top growth.

(2) Slightly lower leaf/stem ratios.

(3) Much later setting of a greater number of tubers which weighe considerably less.

(4) Lower tuber/top ratios (except late in the season with the very late strain 23).

F. Plants grown under northern conditions differed from those under southern conditions in having:

(1) Much greater vegetative growth, vegetative parts eventually having weighed two to six times more.

(2) Much lower leaf/stem ratios.

(3) Many more tubers which appeared 30 to 40 days later on stolons which were much longer.

(4) Low rate of daily tuber increase per plant before midseason and a very high rate during the last few weeks.

(5) A much lower daily increase per gram of dry weight in leaves and consequently very much lower tuber/top ratios.

(6) Higher dry-matter content in vegetative parts, especially as plants became older.

(7) Probably a slightly greater starch content of tubers.

G. Plants grown with a nutrient solution the nitrogen content of which was only 10 to 20 percent of that in a "complete" nutrient solution differed from those grown with the complete solution as follows:

(1) Vegetative growth was greatly restricted. Under southern conditions increase in vegetative weight practically ceased about the fiftieth day, but under northern conditions it continued 4 to 5 weeks longer. Eventually the decrease in vegetative growth was greater under northern than under southern conditions.

(2) Leaf/stem ratios were generally slightly lower.

(3) Tuber setting occurred much earlier under northern but only slightly earlier under southern conditions.

(4) The number of tubers was greatly reduced, especially under northern conditions.

(5) The yield of tubers on all except the first two harvest dates was much smaller under southern conditions, but under northern conditions it was much greater till after the seventy-sixth day.

(6) Tuber/top ratios were higher till the sixty-second day with southern and till after the ninetieth day with northern plants.

(7) The percentage of dry matter was lower in all comparable plant parts.

(8) Starch content of tubers appeared to be slightly lower.

H. Under the light conditions of early spring at latitude and temperatures typical of the extreme Southern States, reduction in nitrogen at the end of 30 days resulted in a slight reduction in vegetative growth and a brief acceleration of tuberization but finally in a great reduction in yield. Increasing the nitrogen content of the nutrient solution from 20 percent to 100 percent (complete solution) resulted in great increases in vegetative growth and tuber production.

DIFFERENTIAL STRAIN RESPONSE

Under practically all conditions the vegetative growth of the very early strain of Triumph potato was slightly greater than that of the very late strain during the first several weeks after emergence. When conditions were favorable for vegetative growth early or throughout the season the growth of the late strain was greater, but if early conditions inhibited vegetative growth there was little difference between strains after the first few weeks. When an external shortage of nitrogen occurred under any conditions total vegetative growth was most extensive and prolonged with the late strain.

Plants of the early strain were always most leafy and compact as shown by higher leaf/stem ratios.

Stolon growth got under way most quickly with plants of the early strain but generally was most extensive with plants of the late strain.

The first tuber formation took place earliest with the early strains. Under southern, short-day, or nitrogen-deficient conditions this difference was much less pronounced than under northern and long-day conditions or with a complete nutrient solution. Prolongation of vegetative growth appeared necessary to enable the late strain to set more tubers than the early strain.

Prior to midseason the weight of tubers was always greatest with the early strains but there was little difference in final yield. When conditions became very favorable for tuberization late in the life of the plants, as under northern conditions or with the application of nitrogen to starved plants, the yields of the late strains were greatest.

Early-strain plants were most efficient in tuber production as shown by higher tuber/top ratios and greater tuber production per day per gram of leaf weight.

On the basis of the results reported in this paper it is evident that periodic harvesting of plants produced under conditions so controlled as to simulate different combinations of environmental conditions is an effective and dependable means of analyzing strains or varieties with a view to determining their probable performance or suitability in certain regions. With a relatively few plants in a comparatively few months it has been possible to discover the essential differences in strains of Triumph potatoes with more certainty than would have been possible in several years of field tests conducted at numerous points in the United States. The results indicate that intrinsic differences between strains are much less evident or important in southern localities where the conditions early in the season are extremely favorable for tuber development than in northern late-potato regions where the plants start their growth in the hot midsummer days.

LYGUS BUG DAMAGE TO ALFALFA IN RELATION TO SEED PRODUCTION [1]

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INTRODUCTION

Investigations have shown that many factors affect seed setting in alfalfa (*Medicago sativa* L.). No fully satisfactory explanation, however, has been found to account for the major declines in yields of alfalfa seed and the seed-crop failures that have occurred in widely separated regions in recent years. Exceptionally high yields, ranging from 400 to 1,000 pounds or more of seed to the acre, have been reported for earlier years of production in the Arkansas Valley of Colorado and in the Uinta Basin and Millard County of Utah (*1, 4*).[3] In 1925 Utah produced 22 million pounds of alfalfa seed, which was approximately 40 percent of the Nation's total of that year. This peak in production was followed by a decline in acre yields and a low total production, which since 1930 has frequently not exceeded 5 million pounds annually.

The general decline in yield of alfalfa seed in Utah appears to be partly attributable to bud damage and flower fall that have recently been shown to result from injury caused by *Lygus* bugs (*Lygus hesperus* Knight and *L. elisus* Van Duzee). *L. elisus* was early recognized by McGregor (*8*) as a pest of cotton in California and as having occurred on alfalfa as an alternate host. The *Lygus* problem in alfalfa-seed production has been studied by Sorenson (*10*), who has contributed a knowledge of the biology and life history of *Lygus* in alfalfa under Utah conditions. Stitt (*11*) likewise has made an investigation of the occurrence and activities of *Lygus* in relation to alfalfa-seed production in Arizona. The present investigation was conducted in an attempt to determine the nature of *Lygus* damage to alfalfa and to obtain evidence of the importance of these insects in relation to a rapid decline in alfalfa-seed production in several formerly highly productive regions of the Western States.

[1] Received for publication July 31, 1940. Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, the Utah Agricultural Experiment Station, and the Wisconsin Agricultural Experiment Station. The material presented here is based on a thesis submitted to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The author gratefully acknowledges the helpful suggestions of O. S. Aamodt, formerly head of the Department of Agronomy, University of Wisconsin (now head agronomist in charge, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture), and other members of the staff of the University of Wisconsin; the assistance of D. C. Cooper, assistant professor of genetics, University of Wisconsin, in the histological studies; and the assistance of Fred R. Jones, senior pathologist, Division of Forage Crops and Diseases; C. J. Sorenson, associate entomologist, Utah Agricultural Experiment Station; L. L. Stitt, junior entomologist, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture; and C. H. Wilkinson, manager, Peppard Seed Co., Delta, Utah. Appreciation is expressed to Eugene Herrling, of the Wisconsin station, and D. F. McAlister, of the Division of Forage Crops and Diseases, for assistance in preparing the photographs.

[3] Italic numbers in parentheses refer to Literature Cited, p. 815.

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TYPES OF BUD AND FLOWER DAMAGE IN ALFALFA

SYMPTOMS OF LYGUS DAMAGE

Severe bud damage and flower fall in alfalfa that are attributable to *Lygus* infestation are indicated in the field by whitish-yellow areas or strips that are conspicuous in contrast to the normal deep green of undamaged and healthy alfalfa. The discoloration results from the presence on the plants of dead, dried, and bleached buds and numerous rachises from which flowers have fallen. The symptoms of damage vary with the age of the alfalfa and with the intensity and time of the infestation. New growth under heavy *Lygus* infestation is often noticeably retarded. Stems are sometimes unusually short and thick and may be terminated by a cluster or rosette of many small and distorted racemes of buds. Heavy and prolonged infestation of older growth may frequently result in an appearance of "stringiness" of the plants, due to excessive branching and leafiness that develop from profuse proliferation. In fields that have been recently irrigated or in areas of a field where the growth for some reason is exceptionally rank and succulent, alfalfa appears to be particularly susceptible to this damage when heavily infested with *Lygus*.

General bud damage in alfalfa is illustrated in figure 1. For comparison, figure 2 shows racemes of healthy buds, flowers, and seed pods that are borne on long peduncles or stalks.

METHODS AND MATERIALS

The nature of *Lygus* damage to alfalfa buds was studied in artificially infested material, with the raceme or cluster of buds taken as a unit. Undamaged specimens were obtained for infestation by tying parchment bags over the tips of stems or branches from which previously formed buds and flowers had been removed, thus permitting the development of new buds in the complete absence of *Lygus*. Racemes of buds from one-eighth to three-eighths of an inch in length were enclosed in sets of four per bag for infestation with either one or two adult *Lygus*, or were exposed to the natural bug population of the seed fields. Check treatments were obtained by enclosing racemes of buds without infestation. The different treatments were applied to racemes of buds on the same plant, and they were repeated with a large number of racemes on several varieties and strains of alfalfa at different times during the seed-setting season. The period of infestation was varied from 20 to 48 hours, after which the insects were released and the bags were replaced to protect the buds from further damage through chance infestation. For evidence of damage based on color, condition, and general appearance, an examination of the racemes of buds was made on the fifth to seventh day after the initiation of the infestations.

A similar procedure was followed in an attempt to determine the effects of *Lygus* bugs on alfalfa flowers, except that the individual flower and not the raceme of flowers was taken as the unit. The flowers on approximately one-half of the racemes were tripped by hand, after which infestation was effected in the manner described for the buds.

Infested and uninfested buds and flowers were preserved in formalin acetic alcohol for histological study. Green and unpreserved material

was useful in a study and description of the general symptoms of damage. Further details of *Lygus* injury could be identified best from a microscopical examination of buds and flowers embedded and stained by ordinary methods. Microtome sections were cut 12μ thick and mounted in serial and chronological order to facilitate tracing the course and rate of spread of the cell disintegration origi-

FIGURE 1.—Terminal portions of three alfalfa stems showing *Lygus* damage. The racemes of buds are small, unusually numerous, crowded, and generally discolored. A tendency to excessive branching and the development of short internodes near the stem terminals is shown. Specimens from an alfalfa field heavily infested with *Lygus* bugs. (Approximately one-half natural size.)

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nating from punctures. Various stains were used. The best resul were obtained with Delafield's haematoxylin and with a combinati of safranin with light green. Details of cell structure and embr development were shown especially well with Heidenhain's iron-alu

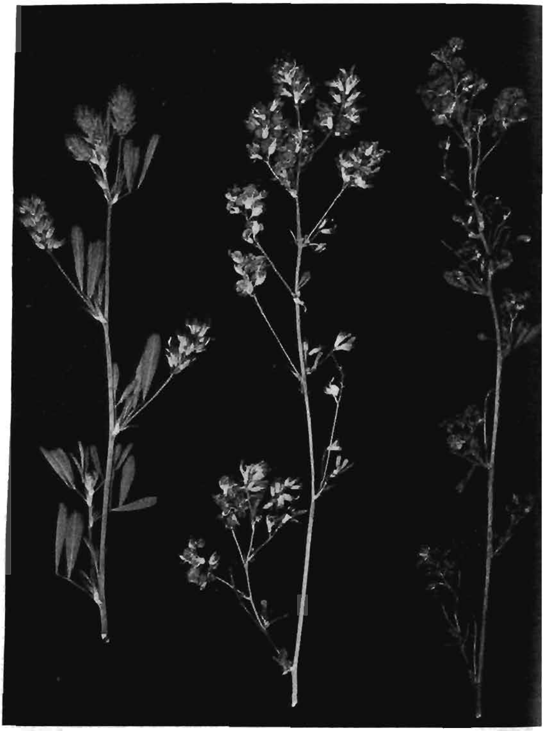


FIGURE 2.—Terminal portions of three alfalfa stems showing an approximatel normal development of buds, flowers, and seed pods. A normal elongation o the internodes and the occurrence of the racemes of buds and flowers on lon peduncles are shown. Crowding of the inflorescences is not apparent and mo of the racemes have a large number of buds and flowers, a large proportion o which develop into seed pods. Specimens from an isolated plant far fro cultivated fields of alfalfa. (Approximately one-half natural size.)

haematoxylin. Generally unsatisfactory results followed a few attempts to trace the origin and course of the damage in buds and flowers without the embedding and staining technique.

NATURE AND EXTENT OF DAMAGE

The initial damage resulting from feeding punctures by *Lygus* is definitely localized. The extent of damage to young buds that may occur under different intensities of infestation is shown in table 1. A single puncture may be seen under the microscope as a small perforation surrounded by a collarlike area of discoloration. (See pl. 1 and fig. 6.) Superficial evidence of damage to buds is indicated by a slight bleaching that becomes apparent 24 to 48 hours after infestation. Death and complete disintegration of the injured buds may follow in a few days. Dead buds frequently remain attached to the floral axis and retain more or less the size and shape they had at the time of injury. Discoloration and disintegration of injured buds begin at the punctures and are apparently caused by a toxic or irritant substance that is emitted with the saliva of the insects at the time of feeding. This observation is supported by the work of Smith (*9*), who studied the feeding methods of capsid bugs (Miridae) which are closely related to *Lygus*. He showed by removing the salivary glands of harmful and harmless species and placing them on apple fruit and foliage and pricking slightly the cells beneath with a fine needle that the damage is chemical rather than physical in character. King and Cook (*7*) showed that *Lygus* bugs and closely related species feed by the same process, namely, by inserting their mouth parts into plant tissue and sucking the sap, but that more serious pathological effects follow feeding by some species and individuals than by others.

TABLE 1.—*Average percentage of racemes of buds apparently normal and of those apparently injured (after 20 to 48 hours' infestation with Lygus bugs* [1]

Infestation	Plants	Racemes	Racemes apparently in condition indicated	
			Normal	Injured
Number of *Lygus* to 4 racemes of buds:	*Number*	*Number*	*Percent*	*Percent*
0	29	116	80.0	20.0
1	17	68	15.6	84.3
2	20	80	13.7	86.2
Natural (field)	25	100	25.0	75.0

[1] Condition of buds determined from evidence of damage based on color and appearance on the fifth to seventh days after infestation began.

The inception and development of *Lygus* damage in alfalfa buds was shown by infesting four normal racemes of buds with four bugs for a period of 12 hours. After the insects were released, the buds were again enclosed and left sufficiently long to allow for the development of different degrees of damage. One raceme of buds was fixed for histological study at the end of the 12-hour infestation period, while others were collected for preparation and study after periods of 24, 36, and 48 hours. In some trials the infestation period was increased by 12-hour intervals up to 60 hours, after which fixation of buds for histological study was made at 12-hour intervals. It was

EXPLANATORY LEGEND FOR PLATE 1

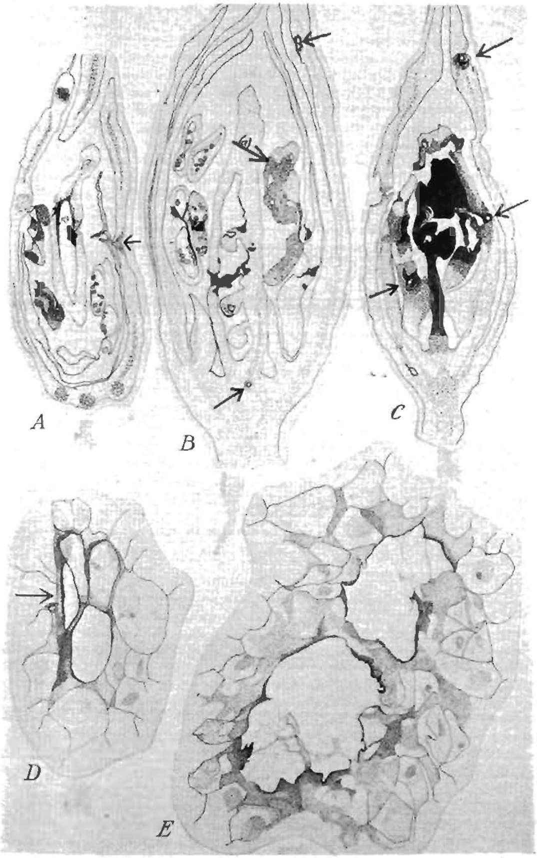
A, Longitudinal section through a young bud from a plant damaged under a natural infestation in the field. The course of a puncture through the floral envelope is shown; it extends into the ovary where an area of cell disintegration has been initiated. (Approximately 42 times natural size.)

B, Longitudinal section through an older bud that was artificially infested with *Lygus*. Localized areas of discoloration and disintegration are shown in which the evidence of punctures has been largely obliterated (*B*, *a*). Recently made punctures are shown near the basal portion of the ovary and in the floral envelope near the apex of the bud. A general disorganization of the bud has resulted. (Approximately 42 times natural size.)

C, Longitudinal section of a young bud that was infested for 48 hours. An advanced stage of disintegration is shown. Arrows point to punctures and centers at which disintegration began. (Approximately 42 times natural size.)

D, Small puncture made by an insertion of the proboscis and showing the early stages of cell discoloration and disintegration. (Approximately 42 times natural size.)

E, Two approximately 24-hour-old punctures showing a comparatively extensive area of disorganization and discoloration. (Approximately 740 times natural size.)



CAMERA LUCIDA DRAWINGS OF ALFALFA BUDS DAMAGED BY LYGUS.

FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

difficult to trace the origin and course in the development of the damage after long infestation, or in material that had developed to advanced stages of deterioration. An infestation of from 12 to 24 hours, with a comparatively short period in which the deterioration might develop, gave evidence that the bud damage had in most cases been initiated from *Lygus* punctures. Extensive disintegration of buds appeared also to have resulted, apparently from the effects of the toxic substance injected into the plants by the insects.

A determination of the nature of *Lygus* damage in alfalfa cannot always be made from material infested in the field. Advanced stages of bud disintegration sometimes obliterate all evidence of the original punctures. Identification, however, can usually be successfully made from artificially infested racemes of buds when sectioned on the microtome, stained, and examined under the low power of the microscope. Numerous punctures made by repeated insertions of the proboscis of the insect have been found within a portion of a single bud. Areas of disintegration absorb the stain more readily than the undamaged parts of buds and can thereby be easily recognized. Mechanical damage resulting directly from *Lygus* feeding punctures is apparently of local and rather limited effect. It appears that major damage develops from secondary or indirect effects, such as distorted vegetative growth and profuse proliferation, which are discussed in detail in a later section of this paper. Recovery from the effects of an infestation of short duration is sometimes noticeable from 5 to 10 days after the application of an insecticide to remove the insects.

BUD ROSETTING

Especially distinctive symptoms of *Lygus* damage to alfalfa result from the development of racemes of buds near the tips of main stems and branches into disklike or knoblike structures called rosettes (fig. 3).

Bud rosetting has been shown to occur under heavy *Lygus* infestation, but this, apparently, is not the only cause of abnormal bud development. The condition is common in Utah alfalfa fields that are setting a poor crop of seed. The compressed state of the racemes of buds appears to result from a failure of the upper internodes of a stem or branch to elongate to the usual length, and from a differential growth in the individual racemes composing the cluster. Racemes of buds in a rosette are, as a rule, small, discolored, and tightly compressed. Frequently most of them fail to produce well-developed clusters of flowers that set seed pods, although considerable variation has been observed in this respect.

Bud rosetting developed in repeated trials on 8 or 10 seedling alfalfa plants from which all previously formed buds and flowers had been removed, when enclosed in cheesecloth cages and infested with 300 late-instar and adult *Lygus*. The rosetting became noticeable on the third day of the infestation when the original number of insects was doubled to increase the severity of the effects. The heavier infestation resulted in a condition that was typical also of field plants outside of the cages. A still further increase in the intensity of the infestation was made at the end of the fourth week by the addition of 150 *Lygus* to the survivors of the original number of bugs in each cage, which resulted in severe and continued damage to the buds for

the remainder of the season. Uninfested plants in check cages did not develop the rosetting, but produced an abundance of flowers from which only a few seeds pods were obtained, owing probably to the unfavorable effects of shading during the long period of enclosure and the failure of the flowers to become fertilized.

The exact nature of bud rosetting has not been determined. Microscopic examinations of rosettes produced under controlled infestation have shown punctures and a bud disintegration generally characteristic of *Lygus* damage. In addition, however, a disturbed growth and development in the stem terminal and bud primordia is apparent. Eggs of insects, which measurements have shown to correspond closely in size and form to those of *Lygus*, have frequently been found in rosettes and in the upper nodes of a stem. Large cavities are also sometimes made in the stem terminals during oviposition, and mechanical destruction of plant tissue may result in some degree. Comparatively little disintegration of cells has been noted surrounding egg cavities. It is possible, nevertheless, that extensive mechanical destruction of cells near the stem tips during oviposition may affect the normal development of structures initiated from the growing point and result as well in a suppressed elongation of terminal internodes.

FIGURE 3.—A suppressed elongation of the apical internodes of alfalfa stems and the development of racemes of buds in clusters called rosettes. The condition occurs under severe *Lygus* infestation. Compare with normal condition (fig. 2). (*Approximately one-half natural size.*)

BUD ABORTION

Damage to alfalfa described as bud abortion occurred in 20 percent of the racemes of buds from which *Lygus* was excluded in the controlled infestation experiments (table 1). Physiological conditions affecting growth and development of the buds, however, appear to be the principal causes contributing to this type of damage. Bud abortion is apparently not unusual, since it has been observed in greenhouse plants and was shown to develop in 21 of 29 field plants on which racemes of buds were enclosed for protection from *Lygus*.

Bud abortion in alfalfa is illustrated in figure 4. The important characteristics are the small size of the racemes of buds and the intense buff-colored bleaching that is conspicuous against the green of healthy stems and leaves. The microscopic character of the bud disintegration and break-down is shown in figure 5, in which it appears to occur uniformly and over the whole raceme at approximately the same time. Damage resulting from bud abortion is distinctly unlike that which develops from *Lygus* infestation. Bud abortion in alfalfa seed fields sometimes complicates the identification of *Lygus* damage, although the two types of damage appear distinctly unlike under microscopic examination.

FLOWER FALL

Flower fall in alfalfa may result from various causes of which insect damage is of great importance. *Lygus* injury to alfalfa flowers is

FIGURE 4.—Bud abortion in alfalfa: not caused by *Lygus* but due to conditions that are unfavorable to bud development and growth. (Approximately one-half natural size.)

shown in sectioned and stained material as punctures and lacerations in the ovaries similar to that occurring in young buds. Damaged individual flowers, however, abscise and are shed so soon after injury that discoloration or disintegration is not conspicuously noticeable while they remain attached to the plant. Excessive flower fall in alfalfa occurred under controlled *Lygus* infestation when either 3 or 5 bugs were enclosed with 7 to 10 flowers for 48 hours (table 2). The percentage of flowers falling was about the same with artificially tripped flowers as with those under natural development, although a higher percentage of the surviving tripped flowers set seed pods. Numerous observations have shown *Lygus* bugs to be an important cause of excessive flower fall in alfalfa seed fields, although these insects are by no means the only cause of low yields and poor seed crops.

Alfalfa is highly variable in its reproductive behavior and is sometimes known to shed its flowers in the apparent absence of destructive

...sects. Brink and Cooper (*2*) have shown that the degree of effective ...llination is very commonly a limiting factor for seed production. ...ooper et al. (*6*) have demonstrated that a failure of seed setting in ...falfa where the flowers have been tripped to insure effective pollina-...on is apparently due to lack of fertilization even though pollen tubes ...re present. The pollen ...ubes sometimes fail to ...ach all the ovules, and ...ven when fertilization ...s effected, embryo abor-...ion may occur at vari-...us stages in develop-...ent. More recently ...ysdal (*12*) has shown ...he importance of trip-...ing insects, particu-...arly wild bees of *Nomia* ...nd *Megachile* species, ...s pollinators of alfalfa ...lowers, and he has ...hown that the presence ...f these insects in alfalfa ...eed fields is frequently ...orrelated with satis-...actory yields of seed.

The nature of *Lygus* ...amage to alfalfa ...lowers is illustrated in ...igure 6. A puncture ...as been made through ...he floral envelope and ...nto the lower extremity ...f the ovary. A col-...arlike area of discolora-...ion surrounds the per-...oration. The originally ...mall area of injury has ...ecome enlarged and ...as resulted in a partial ...isintegration of the ...ower one-third of the ...vary. A generally ex-...tensive area of damage ...s shown within the ...ovary and appears to have destroyed about one-half of the ovules. Most of this damage appears to be traceable to the effects of a single puncture.

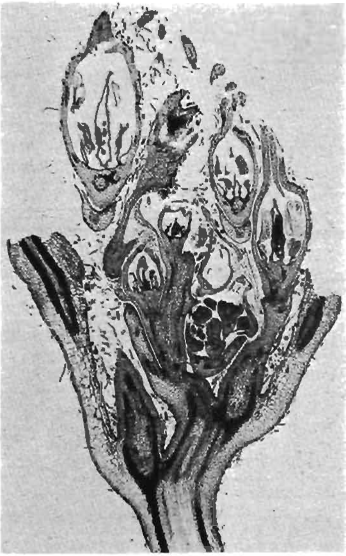


FIGURE 5.—Photomicrograph of median longitudinal section through a raceme of young aborted alfalfa buds. The break-down and disintegration of the individual buds is general and uniform over the whole raceme, in contrast to the localized disintegration that follows *Lygus* damage. (Approximately 25 times natural size.)

A condition of alfalfa flowers that fall from causes other than insect damage is illustrated in figure 7. A shriveling and deterioration of the ovules occurs without evidence of punctures, lacerations, or other mechanical injury, and with no indication of a deterioration due to toxic effects. The appearance of the ovules suggests a failure in development due to a lack of fertilization, or an embryo abortion

following fertilization that has resulted in the development of shrivele seeds.

TABLE 2.—*Average percentage of normally developed and of artificially trippe alfalfa flowers that fell while exposed to an infestation of Lygus bugs for 48 hou and average percentage of the total flowers that formed seed pods within 10 da after infestation period*

Infestation [1]	Plants	Flowers of indicated treatment falling duri infestation period and those setting seed po within 10 days after infestation [2]			
		Normal development		Artificial tripping	
		Flowers falling	Setting seed pods	Flowers falling	Setting seed pod
Number of *Lygus* per 7 to 10 flowers:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
0	40	9.3	10.3	5.8	52
1	15	32.1	4.4	32.1	26
2	15	36.0	4.2	30.4	9
3	5	80.8	2.1	64.6	2
5	5	97.9	0	89.1	
Natural (field)	40	6.9	22.5	9.4	3

[1] Adult *Lygus* enclosed with flowers in paper bags; released at the end of 48 hours, after which the ba were replaced over the flowers.
[2] Remainder of the flowers not forming seed pods fell after the infestation period.

SECONDARY EFFECTS OF LYGUS DAMAGE IN ALFALFA

METHODS AND MATERIALS

The pathological symptoms appearing in the buds and the damag suffered by alfalfa plants infested with *Lygus* bugs led to inquir regarding possible physiological disturbances in growth and repro ductive development that might impair the plants for seed settin *Lygus* infestation and insecticide treatments were applied to tran planted, hill-spaced, alfalfa seedlings of Grimm, Hardistan, Kansa Common, and a heavy seed-producing strain of a Wisconsin Grim selection. The plants of the different alfalfas were spaced 3 by 3 fe apart in random order and within partly isolated blocks a rod squar The special purpose of the isolation was to eliminate the drifting of th insecticide from treated to untreated plots during application.

Treatments were begun on June 20, when all plants were in th prebud stage of development and approximately 8 inches hig Infestation was effected by using cheesecloth cages to confine th *Lygus* bugs to the plants for a period of 24 hours. Once establishe the infestation was maintained by adding to each plant, from time t time, the number of bugs necessary to produce a damage approx mately as severe as that occurring naturally in nearby alfalfa field Prevention of damage by the insects on control blocks was effected b the frequent application of a dry pyrethrum concentrate containing percent pyrethrins, in the proportion of 15 parts to 85 parts of collo dal or electric dusting sulfur as a carrier.

Variations in the rate or frequency of application of the insecticid were not made, since a maximum control of the insects and a preve tion of the damage was all that was desired. Infestation and contr treatments were applied to blocks in duplicate as follows:

Treatment 1.—Infested with *Lygus* bugs from the prebud stage to the full-bloom stage of development. Dusted after the full-bloom stage five to seven times weekly until seed harvest. Objective: To determine the effects of *Lygus* infestation during the early vegetative stages of growth and development of alfalfa, and the later effects on seed setting.

Treatment 2.—No infestation; dusted five to seven times weekly from the prebud stage of development of the plants to seed harvest. Objective: To determine the type of growth and development in alfalfa and the effects on seed setting when plants are comparatively free from *Lygus* infestation.

Treatment 3.—Same as for 2, except the early buds and flowers were clipped by hand to approximate the damage resulting from *Lygus* infestation under treatment

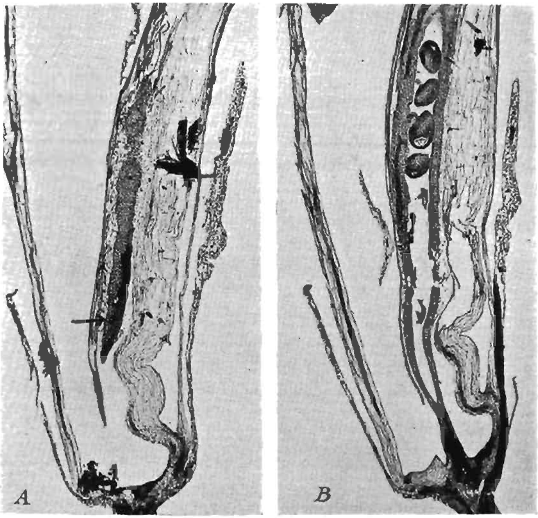


FIGURE 6.—Photomicrograph showing the effects of a *Lygus* puncture in the ovary of an alfalfa flower. *A*, Oblique longitudinal section exposing the ovary and showing a puncture that has resulted in a discoloration of the basal one-third of the ovary. *B*, Median longitudinal section from the same flower, exposing the ovules and the area of damage within the ovary. The localized nature of the damage is shown. The ovules in the upper one-half of the ovary are apparently free from injury.

1. Objective: To determine the effects on growth, development, and seed setting in alfalfa when the loss of buds and flowers occurs from causes other than *Lygus* damage.

Treatment 4.—Infested continuously with *Lygus* from the prebud stage of growth and development until seed harvest. Objective: To determine the effects of continuous *Lygus* infestation on alfalfa, with special reference to effects on seed setting.

Treatment 5.—Uninfested and dusted from the prebud stage to the full-bloom stage of development. Thereafter, infested with *Lygus* until seed harvest. Objective: To determine the effects of *Lygus* infestation during the bud and the flower stage of alfalfa development in relation to seed setting.

EFFECTS OF LYGUS INFESTATION ON EARLY GROWTH AND DEVELOPMENT OF ALFALFA

The average height of the plants in the various blocks previous t infestation ranged from 6.9 to 9.1 inches. Fifteen days after infesta tion the infested plants had an average height of 12.3 inches, as com pared with an average height of 17. inches for uninfested plants that wer dusted frequently. At the end of 2. days, when the plants were in ful bloom, the average heights were 14.. and 21.3 inches for infested an dusted plants, respectively. Th effects of the two treatments o growth of the plants are illustrated i figure 8. The photographs wer taken to the same scale to show thes effects directly.

FIGURE 7.—Photomicrograph of a median longitudinal section of an alfalfa flower that has fallen from a raceme from a cause not directly attributable to *Lygus* injury. A shriveling and deterioration of the ovules is shown, with no apparent damage to the floral envelope or the ovary. The condition is typical of a large percentage of dehiscing alfalfa flowers and may result from failure of pollination, lack of fertilization, or embryo abortion.

Infested and dusted plants, wit the exception of those in one block developed approximately the sam number of main stems (table 3). A average of 40.8 branches develope on 3 main stems of infested plants, a compared with an average of 25. on the uninfested and dusted plants Wide variations in the tendency t profuse branching was shown b plants within blocks receiving th same treatment. Differences in re sponse to infestation shown by th plants are probably due, at least i part, to a difference in the intensit or duration of the infestation occur ring with individual plants within th same block. Excessive branching which gives to affected plants an ap pearance of "stringiness," seems t result from a stimulation into activ ity of normally dormant axillar branch primordia due to *Lygus* injur at apical growing points of mai stems and branches. Infested an uninfested plants developed approx imately the same number of leave on 3 main stems. The appearanc and condition of the leaves, however, was strikingly different. A average of 21.8 percent of leaves from infested plants were crinkle or misshaped, as compared with 1.6 percent of those from the duste

plants. Attempts to produce the leaf deformity by exposing young leaves to direct attack by the insects did not give rise to characteristic symptoms. It is possible that injury may occur indirectly to leaf-bud primordia and this results later in the development of crinkled and deformed leaves. An objection to this view arises from the fact that *Lygus* damage to young flower buds, which are probably less sensitive

FIGURE 8.—Effects of *Lygus* infestation on the early growth and development of alfalfa plants: *A*, Infested from prebud stage to full-bloom stage of development; *B*, Uninfested and dusted five to seven times weekly for the same period.

to injury than leaf-bud primordia, results in immediate and complete deterioration. Nevertheless the variability in the number and proportion of crinkled and deformed leaves on plants within the same block and on different blocks and the tendency to profuse branching, as well as crinkled leaves, suggest *Lygus* as a direct cause.

TABLE 3.—*Comparative growth and development of alfalfa when infested at the prebud stage of development with Lygus bugs and when uninfested and dusted 5 to 7 times weekly with an insecticide*

Treatment	Growth and development of alfalfa [1]						
	Average height			After 15 days' treatment			
	Before treatment	After 15 days	After 22 days	Average stem per plant	Average branches per stem [2]	Average leaves per stem [1]	Crinkled leaves
Infested with *Lygus*:	*Inches*	*Inches*	*Inches*	*Number*	*Number*	*Number*	*Percent*
Block A	7.1	11.3	14.0	13.1	34.1	79	32.9
Block B	9.0	13.3	15.0	13.6	47.6	121	10.7
Mean	8.0	12.3	14.5	13.3	40.8	100	21.8
Dusted 5 to 7 times weekly:							
Buds not clipped:							
Block A	7.5	15.7	21.4	13.0	20.8	101	1.9
Block B	9.1	19.0	22.8	12.8	27.8	108	.9
Mean	8.3	17.3	22.1	12.9	24.3	104	1.4
Buds clipped:							
Block A	6.9	16.1	19.6	13.0	21.3	102	2.9
Block B	8.8	18.1	22.0	9.8	32.3	120	.8
Mean	7.8	17.1	20.8	11.4	26.8	111	1.8

[1] 16 plants per block. [2] 3 main stems.

DEVELOPMENT OF PLANTS FOLLOWING REMOVAL OF LYGUS

The *Lygus* bugs were removed effectively from infested blocks after repeated and frequent application of the insecticide. Evidence of a general recovery from the effects of a relatively brief infestation also became noticeable 10 days after the removal of the insects. Growth occurring subsequent to removal of the insects gave an appearance of having been superimposed on the previously damaged stems and branches. The first buds and flowers to develop after the removal of the insects appeared on short branches, as shown in figure 9. Later, longer stems and branches developed which had internodes of the usual length and buds and flowers borne on long peduncles, as is characteristic of normal and healthy alfalfa (figs. 10 and 2).

No marked effects resulted from clipping of the early formed buds and flowers under treatment 3. Neither were harmful effects observed to follow the use of the insecticide, at this stage of the investigation. Growth and floral development of frequently dusted plants were thought to be generally good and were apparently normal in all respects. Buds and flowers were produced in the abundance and succession characteristic of alfalfa growing under favorable conditions. Later it was observed that applications of insecticides having sulfur as a carrier resulted in a slight burning of the flowers, which damage has since been shown to result in reduced yields of seed.

EFFECT OF LYGUS INFESTATION IN THE FULL-BLOOM STAGE OF ALFALFA

Lygus infestation initiated in the full-bloom stage of alfalfa resulted in damage to buds and flowers and a condition similar to that commonly observed in commercial seed fields when producing a poor crop of seed. Discolored buds and an excessive flower fall gave early evidence of damage, while rosetting developed as a later effect.

Microscopic examination of injured buds showed characteristic *Lygus* punctures similar to those obtained under more closely controlled infestation. Growth and development of the plants were not retarded by late infestation. The usual "stringiness," however, gave to the plants an appearance of excessive vegetative development. In general flowers were produced abundantly, although most of them

FIGURE 9.—Stem from a *Lygus*-infested alfalfa plant showing distorted development and a tendency to excessive branching. Racemes of buds, flowers, and seed pods that are apparently normal (fig. 2) developed on short branches soon after the removal of the infestation by frequent dusting.

abscised early and before seed pods were formed. The production of seed was also generally poor.

EFFECTS OF LYGUS INFESTATION ON SEED AND FORAGE PRODUCTION

Seed yields from plants receiving different infestation and insecticide treatments differed strikingly (table 4). The highest yields of seed per plant resulted from uninfested plants that were dusted frequently

to control *Lygus*, while the lowest yields were obtained from those undusted and continuously infested. Differences in the yield of seed resulted also from variations in the time of applying the infestations to plants in different stages of vegetative and reproductive development. Generally good agreement in yields is shown by replicates of the various treatments, which is evidence of a satisfactory control of *Lygus* under the conditions of the experiment.

FIGURE 10.—Type of growth and reproductive development on severely damaged alfalfa plants following the removal of a *Lygus* infestation. Below the line: Retarded and distorted stem development and excessive branching that is typical of the effects of *Lygus* on early growth and development of alfalfa, except two branches at the extreme right. Above the line: Growth and development that followed the removal of the infestation. Note the normal elongation of the stem internodes and the development of racemes of buds and flowers on long peduncles, as is the normal condition in alfalfa (fig. 2).

Mean yields of slightly more than 12 gm. of seed per plant, which were obtained from the uninfested and dusted plants, are estimated to be equivalent to an acre yield of from 200 to 300 pounds. This estimate is based on the yields of many 1-year-old hill-spaced alfalfa plants at the Uinta Basin Alfalfa Seed Experimental Farm of the Utah Agricultural Experiment Station in years when successful seed crops were produced. The approximation is subject to some error owing to differences in the degree to which young hill-spaced alfalfa plants utilize effectively and efficiently the space allotted to each, but is, nevertheless, of value as a means of providing some idea of the beneficial effects that might be expected from a control of *Lygus*. The low average yield of 2.30 gm. of seed per plant obtained from the plots infested with *Lygus* would not, on the same basis of comparison, be expected to pay for the costs of harvesting and threshing.

The differences in yield of seed shown by infested and insecticide-treated plants are highly significant statistically. Differences resulting from varying the time of the infestation in relation to stages in the development of the plants are small but near the border line of low statistical significance. The consistently low yields of seed obtained under continuous *Lygus* infestation are evidence that greater damage to seed production results from long and continued *Lygus* infestation than from short or

periodic infestations of limited duration. More serious damage to seed production seems to have resulted from *Lygus* infestation in the full-bloom stage than from infestation in the early stages of vegetative development. Infestation at any time, however, may be expected to result in some damage. Clipping the early buds and flowers of uninfested and dusted plants, as in treatment 3, produced no statistically significant effect on seed production as compared with treatment 2, in which they were allowed to grow and to which the same insecticidal treatment was applied.

TABLE 4.—*Average yield of seed in grams per plant of 16 alfalfa plants (per block) spaced uniformly in square rod blocks when infested with Lygus bugs at various stages of growth and development and when uninfested and dusted 5 to 7 times weekly with an insecticide*

Treatment of alfalfa plants [1]	Yield and quality of seed			
	Average seed per plant			Average proportion of good seed
	Block A	Block B	Mean	
	Grams	*Grams*	*Grams*	*Percent*
1. Infested during the prebud stage and dusted in later bloom stages	5.80	8.35	7.07	75.8
2. No infestation; frequent dusting	11.85	10.13	10.99	83.0
3. Same as 2 (early buds clipped)	12.51	14.85	13.68	80.2
4. Continuous infestation	3.15	1.46	2.30	48.6
5. Dusted during the prebud stage and infested in bloom stage	2.91	4.25	3.58	63.9
Significant difference (5-percent point)			3.59	18.5

[1] For complete description of treatments, see p. 803.

Significant differences were obtained in the quality of the seed produced under continuous *Lygus* infestation and that produced under frequent insecticide treatment (table 4, last column). *Lygus* is, therefore, shown to affect the quality and value of alfalfa seed, as well as to damage buds and flowers. A high shrinkage in recleaning, which in some cases exceeded 50 percent, has occurred in commercially produced crops in years when *Lygus* has been suspected as a cause of the low yields.

The average weights of air-dry forage produced by the alfalfa plants under different infestation and insecticide treatments are given in table 5. Generally good agreement in some cases and wide differences in others are shown between the mean weights of the plants in the two replicates of the treatments. A statistically significant difference is shown between the mean weight of the plants under continuous *Lygus* infestation and that of the plants in the other treatments. The data are probably insufficient to permit definite conclusions as to the effects of *Lygus* on the vegetative development of alfalfa. General experience and observation in the production of alfalfa seed under *Lygus* infestation have shown that on good soil and with a not too restricted water supply, vegetative growth sometimes becomes excessive to the extent that cutting and threshing of poor seed crops becomes difficult and costly. It is not known definitely, however, whether this excess growth results from a failure of the plants to set seed or from a stimulation due to *Lygus* injury (fig. 11).

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FIGURE 11.—Comparative growth and vegetative development of *Lygus*-infest and uninfested alfalfa plants: *A*, Continuously infested from prebud stage development to seed harvest (treatment 4); *B*, uninfested and dusted five seven times weekly for same period (early buds and flowers clipped; treatme 3). Photographs taken to the same scale. Compare the ground covered l the plants for an idea as to their relative size.

TABLE 5.—*Average weight of air-dry forage in grams per plant of 16 alfalfa plants spaced uniformly in square rod blocks when infested at various stages of growth and development with Lygus bugs and when dusted 5 to 7 times weekly with an insecticide*

Treatment of alfalfa plants [1]	Yield of air-dry forage		
	Average weight of air-dry forage per plant		
	Block A	Block B	Mean
	Grams	*Grams*	*Grams*
1. Infested during the prebud stage and dusted in late bloom stage	215	217	216
2. No infestation; frequent dusting	192	214	203
3. Same as 2 (early buds clipped)	204	209	206
4. Continuous infestation	254	259	256
5. Dusted during the prebud stage and infested in bloom stage	207	237	222
Significant difference (5-percent point)			29

[1] For complete description of treatments, see p. 803.

DISCUSSION AND CONCLUSIONS

It is in recent years only that attention has been given to *Lygus* bugs as pests in alfalfa seed production. While these insects are known to be widely distributed and to feed on a variety of native and cultivated host plants, comparatively little is known of their occurrence in alfalfa seed fields prior to the general onset of alfalfa seed crop failures in Utah. However, evidence gained from a study of the biology, life history, and general habits of *Lygus* in relation to alfalfa seed production (*10*); their occurrence and the intensity of the infestations in relation to alfalfa seed setting (*11*); and the nature of the damage, as determined in the present investigation, leaves little doubt as to the great importance of *Lygus* as a factor in alfalfa seed production at the present time. Since the *Lygus* species damaging alfalfa are native to western America, it is highly probable that they have been present in the alfalfa seed fields of the Western States for many years. It is not clear, however, as to how, when, and why they have apparently only recently become sufficiently numerous to constitute a pest of prime importance.

Brues (*3*) has shown that insects sometimes change their food habits and are known to leave their wild host plants and adopt new food plants among cultivated crops. Two weeds of the family Chenopodiaceae that are known to harbor *Lygus* in large numbers have become widely distributed in Utah alfalfa seed-producing areas since the land was first brought into cultivation. The best known and most widely distributed is Russian thistle (*Salsola pestifer* A. Nels.) and the other is smother weed (*Bassia hyssopifolia* (Parl.) Kuntze). *Chenopodium* species were early reported by McGregor (*8*) as the most common of the native host plants of *Lygus elisus* in the Imperial Valley of California. Winslow et al.[4] reported declining yields of alfalfa seed as beginning with the advent of sugar-beet seed growing in the Hemet Valley of California, which is significant in view of the fact that *Lygus* is also recognized as a pest of sugar-beet

[4] WINSLOW, E. M., HUBERTY, MARTIN R., and STITT, LOYD L. PROGRESS REPORT SURVEY OF ALFALFA SEED, HEMET VALLEY. Agr. Ext. Serv., Riverside, Calif. 8 pp. 1938. [Mimeographed.]

seed production in Utah by workers in the Division of Sugar Plan Investigations of the Bureau of Plant Industry, United States De partment of Agriculture. Furthermore, somewhat limited experienc by the Division of Forage Crops and Diseases in growing seed o improved strains of alfalfa in Utah has shown severe *Lygus* infesta tion and damage to develop on blocks adjacent to sugar beets. Les damage has been observed on blocks adjoining grassland, winte wheat, and fallow. Various conditions and complex ecological rela tionships may, therefore, be given consideration in an attempt t explain why *Lygus* has reached its present status as a major pest i alfalfa seed production.

Complete information is lacking also as to the conditions unde which marked declines in yield of alfalfa seed have occurred. Cer tain striking similarities may be noted in the general sequence o events that have led to low yields of alfalfa seed in several widel separated regions. Production began in western regions, as a rule on virgin soil and with a comparatively small acreage. The yield in the early years of production were often high, ranging from 400 t 1,000 pounds or more of seed to the acre. A peak in total productio resulted, followed by declining yields and a low total production While new lands were available, seed-producing areas were develope rapidly in new regions and seed growers continued production wit profit for many years in succession. At the present time, certain o these same regions are characterized by frequent seed-crop failures low acre yields, and generally low total production of alfalfa seed.

A few acres of alfalfa were planted on virgin land in the bottoms o Wah Wah Valley on the western desert of Utah in the summer of 1936 The severe bud damage and flower fall that are typical of *Lygu* injury were observed in the area the following year. Sweepings wit an insect net showed the *Lygus* population to be as high as that i representative fields in the older alfalfa seed-producing regions o Utah and Idaho. The observation is of special interest because of th fact that the planting of alfalfa was the first to be made in the regio and no other alfalfa fields were known within a radius of approximatel 30 miles. The infestation had evidently developed through migratio of *Lygus* from the native host plants of the desert. On the othe hand, some outstandingly successful seed crops have been reported from fields bordering on the desert during a period of seed-crop failures in older seed-producing areas in Utah.

Carlson and Stewart (*5*) and Carlson (*4*) reported yields of alfalfa seed from experimental trials that ranged from less than 50 to more than 800 pounds of seed to the acre, depending upon treatments, varieties, soils, and seasons. The observed symptoms of damage to the buds and the excessive flower fall associated with some of the low yields seem to afford unmistakable evidence that *Lygus* damage, as it is now known, was present in the fields, and the insects were probably an important cause of the poor seed setting. Plots that produced maximum yields in one year frequently gave exceptionally low yields the following year and vice versa. Wide variations in yields occurred among plots receiving similar treatments in the same year and in different years. It has been shown (table 4) that severe *Lygus* infestation in the full-bloom stage of alfalfa resulted in greater damage to the seed crop than did infestation in earlier vegetative stages of

ılant development. Yield fluctuations among plots receiving the ame cultural treatment are, therefore, at least in part, probably xplainable on the basis of differences in the severity and time of ccurrence of *Lygus* infestations in the various plots.

Fluctuations in yield of alfalfa seed in commercial fields are freuently as great as those reported for experimental plots and seem to e attributable to the same general causes. Yields of alfalfa seed eported to exceed 800 pounds to the acre were being produced on cattered and isolated ranches on the western desert of Utah only 0 miles distant from the areas of outstanding seed-crop failures in Iillard County in 1934. Generally high acre yields of seed and high otal production were obtained in northern Utah and southern Idaho or several years when seed-crop failures occurred in the formerly highly roductive areas of Millard County and the Uinta Basin of Utah. Iore recently, a decline in production has been noted in certain egions of northern Utah and southern Idaho, while a marked improvement in conditions favoring seed setting in alfalfa has become pparent in portions of Millard County. These wide fluctuations in eed yields within relatively small geographical areas seem to offer nmistakable evidence of the effects of local factors rather than general limatic changes. In addition, symptoms of damage attributable to *Lygus* and the presence of the insects in seed fields at critical periods in he development of the seed crops are strong evidence that these bugs re in some way responsible for the low yields of seed, especially when onditions are otherwise apparently favorable for seed production.

CONTROL OF LYGUS

Sorenson (10) reported that the application of insecticides for the control of *Lygus* on a large scale is insufficiently effective to justify he cost and that the bugs possess a high natural resistance to most nsecticides now available. However, in the present investigation, the frequent application of pyrethrum concentrate and sulfur to small plots resulted in considerable improvement in seed production, but particularly in the condition of the buds and flowers previous to pod setting. The average yields of seed per plant on 10 seed-increase plots that were dusted frequently during the growth of the seed crop in 1938 were approximately 10 times those from similar plots in 1937 when the insecticide was not applied until after severe bud damage and flower fall had occurred. The improved yields resulting from the use of the insecticide in 1938 are especially significant in view of the fact that the alfalfa seed crop of that year from the commercial fields in the region in which the trials were conducted was one of the poorest on record.

Increased yields of alfalfa seed have resulted in some cases from various cultivation and sanitation practices attempted by commercial seed growers. Some of these methods have not been tested under experimental control in Utah, and recommendations cannot, therefore, be made at this time. In one case, a *Lygus*-infested alfalfa field in which dry grass was burned following the removal of the first hay crop showed a striking improvement in the appearance and condition of the buds and flowers on the subsequent growth of the alfalfa. The seed produced after the burning was reported to have exceeded 200 pounds

to the acre and was the best that had been produced in the field for several years. It was obvious from inspection that this seed crop was superior to those in adjoining unburned fields. Owing to prolonged drought during the spring of the following year, the grass was too scanty to make burning an effective method of destroying the insects in the field, and it was not therefore attempted by the seed grower. The hay, however, was cut at the usual time, and the treatment of the field was the same as in the previous year except for the burning of the grass. The effect was that the alfalfa became heavily infested with *Lygus*, severe damage was caused to the buds and flowers, and a generally distorted development of the plants was in evidence. The seed crop was generally poor and was reported as not exceeding 100 pounds to the acre.

SUMMARY

An investigation was made to determine the effects of *Lygus* bugs (*Lygus hesperus* Knight and *L. elisus* Van Duzee) on alfalfa (*Medicago sativa* L.) in relation to seed setting, and to gain evidence of the extent to which these insects have been a factor contributing to a rapid decline in alfalfa seed production in several formerly highly productive seed-growing regions of the Western States.

Several types of bud damage are found in alfalfa. A mechanical localized damage has been shown by controlled infestation to result directly from punctures and lacerations made by the mouth parts of feeding *Lygus* bugs, although pathological effects develop indirectly from the initial damage caused by the insects. Damaged buds show discoloration and evidence of deterioration in from 24 to 48 hours after injury. A rapid disintegration of the buds that apparently results from a toxic substance emitted with the saliva of the feeding insects follows injury.

Rosetting has been shown to occur under heavy *Lygus* infestation and is characterized by the development of racemes of buds near the tips of main stems and branches into disklike or knoblike clusters.

Bud abortion, another type of damage occurring in alfalfa, is apparently not attributable to *Lygus* infestation, but rather to physiological factors and conditions unfavorable to bud growth and development.

Individual alfalfa flowers abscise and are shed soon after injury by *Lygus*. Damage to flowers results mainly from punctures in the ovary or other succulent parts. However, all flower fall in alfalfa is not attributable to insect damage, but may be due also to factors affecting fertilization and embryo development.

Lygus injury apparently also affects the vegetative growth and development of alfalfa. Young growth when heavily infested is definitely retarded, and shows a tendency to excessive branching. A high proportion of the leaves of heavily infested plants may be crinkled or deformed.

Changes in the character of vegetative growth and reproductive development of alfalfa plants follow removal of a *Lygus* infestation. Evidence of recovery from damage usually becomes noticeable within 10 days.

Bleached and discolored buds give early indications of *Lygus* damage to alfalfa infested in the full-bloom stage of development. Rosetting

develops as a later effect. Vegetative growth of alfalfa is not retarded by late infestation, but severe damage to the buds and flowers may result.

Uninfested plants that received frequent applications of an insecticide gave significantly higher yields of seed than did infested plants. Infested plants, however, gave a significantly higher yield of air-dry forage, but it is not known whether the increased yield of forage resulted from the effects of *Lygus* injury or from a failure of the plants to set an abundance of seed. Numerous observations made during survey studies to determine the cause of alfalfa seed-crop failures have shown serious damage to alfalfa to result more or less directly in proportion to the *Lygus* population of the seed fields. *Lygus* bugs are, therefore, regarded as an important cause of the major alfalfa seed-crop failures in Utah.

The importance of *Lygus* as a factor affecting alfalfa seed production in Utah is evident also from the nature of the damage to the buds and flowers, and by the significant improvement in the yield of seed that is consistently obtained when *Lygus* bugs are effectively controlled.

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TOXIC EFFECT ON GERMINATING SUGAR-BEET SEED OF WATER-SOLUBLE SUBSTANCES IN THE SEED BALL[1]

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INTRODUCTION

Differences among sugar-beet varieties in the rate of seedling emergence are commonly observed. These varietal differences are generally more pronounced under laboratory conditions than in the field and may be apparent for a long or short period of time, depending upon the varieties under observation.

Results[2] obtained by C. H. Smith, of the United States Sugar Plant Field Laboratory at Salt Lake City, Utah, had shown that washing the seed balls in water prior to planting accelerated germination and that water alone was as effective as any of an extensive series of salt solutions at various concentrations. These results are in line with the findings of Stehlík and Neuwirth (*18*),[3] who concluded that a preliminary soaking in water was equal to any other seed treatment to which stimulative action had been attributed. No suggestion was made as to what was accomplished by washing or presoaking of seed. The rather inconclusive and contradictory findings on the debated issue of seed stimulation are reviewed by these authors, who challenge the evidence on seed stimulation.

The presence of substances in the seed or seed coat of several seeds that either inhibit or stimulate germinating seeds has been reported. Axentjev (*1*) reported that the inhibiting action of seed extracts is not dependent upon fluorescence, and that seed extract of *Phacelia* may inhibit germination before it is exposed to light. He stated that the action of *Phacelia* seed extract was not specific but that it inhibited the germination of some seeds and stimulated others. Mosheov (*12*), in reporting his work relative to the presence of germination-inhibiting substances in wheat, stated that the substances are not heat stable and that the inhibiting effect is greater in light. Lehmann (*10*) reported the presence of heat-labile, germination-inbibiting substances in the hulls of buckwheat seeds.

Juices from fleshy fruits have long been known to retard or inhibit the germination of seeds. Oppenheimer (*13, 14*) first postulated the presence of specific inhibitors in fruit juices in addition to the osmotic effect. He was later supported in his conclusions by Fukaki (*5*), Reinhard (*15*), and Köckemann (*8*). Lavialle (*9*) and Litvinov (*11*) claimed that the inhibitory action was almost entirely due to the osmotic pressure of the fruit juices.

The germination of sugar-beet seed may be influenced by several factors, such as vigor of the embryo, seed-ball structure, and the environmental factors imposed. That substances may be present in the seed ball that either hasten or retard germination seems not to

[1] Received for publication September 3, 1940.
[2] Unpublished.
[3] Italic numbers in parentheses refer to Literature Cited, p. 829.

have been heretofore considered. The experiments herein reporte relate to the toxic effect on germinating sugar-beet seed of wate soluble substances in the dried fruit of the sugar beet, and the relatio ship of these effects to laboratory procedure in germination tests.

MATERIALS AND METHODS

Four sugar-beet varieties developed in the curly top-resistan breeding program, Nos. 5194, 550, 523, and 68, were used in the studies. According to the experience at the Salt Lake City labor tory, variety 5194 germinates extremely rapidly, whereas variety (germinates so slowly that it has been a rather common practice plant it a week in advance of some other varieties when relative uniform emergence from the soil was desired. The other two varieti are intermediate in germination rate.

In commercial usage, the term "sugar-beet seed" refers to tl glomerules made up of the matured flowers of the beet. The glomerul or seed ball, may contain from one to several true seeds. In the experiments, comparisons of rate and total germination were ma with seed balls and the naked seeds freed from the surrounding e velope. The naked seeds were obtained readily in quantity by grin ing the seed balls in a hand coffee mill set to grind coarsely. Uni jured seeds freed from all pericarp tissue were picked out from tl broken seed balls. For convenience, in this paper the term "pe carp" is used to designate all parts of the seed ball that are not see and includes the ripened ovary wall and closely related parts, such the base of the perianth and the receptacle.

Two points of procedure proved to be rather crucial in the prop evaluation of factors influencing the germination of sugar-beet see By the use of both seed balls and naked seed it was possible to compa the germination of the true seed in connection with and independe of its pericarp. The use of a thin cotton substratum in Petri di germinators prevented the leaching of water-soluble substances fro the pericarp and also made it possible to see the seeds at all time On several occasions striking differences were observed in the cond *tion and rate of growth of sprouts, which indicated further dama* than that shown by the number of seeds that failed to germinate.

Water-soluble substances from intact seed balls and from perica tissue were similar in their effect on the germination of naked seed The extracts were obtained by soaking seed balls at room temperatu for 20 hours in five times their weight of distilled water, and filteri the liquid through filter paper. The cotton substratum in the Pet dishes was then moistened with 9 cc. of the filtrate, after which 5 naked seeds of variety 68 were placed in each dish. The lids we then replaced and the dishes held in the dark at approximately 24° (The decrease in rate and total germination of naked seeds in the Pet dish when water extracts from seed balls were used, as compare with results obtained when distilled water was used, was taken the measure of the toxic effect of each extract. Throughout the tes of extracts, naked seeds of variety 68 were used because they we uniform and easier to obtain. Repeated tests had shown that nake seeds of other varieties gave entirely similar responses.

From two to four replicates were used for each variety or treatme and the data were analyzed by Fisher's variance method (*3*). A analysis was made on the data obtained at each germination cou

nd also on the combined data, including all hours of count for each periment. This made it possible to measure the varietal or treat-ent differences in both rate and total germination. In any single periment there was not a significant difference between the errors r the separate hours of count. In view of this, the general error rived from the combined analysis of all periods of count was used. he data for each hour of count are taken from the same Petri dishes, the analysis is very similar to a main and subplot arrangement (*4*). The analysis of the data in table 2, for example, was keyed out as own in table 1.

ABLE 1.—*Analysis of variance of germination of intact seed balls of four varieties of sugar beets including three periods of count*

Source of variation	Degrees of freedom	Mean square	Calculated *F* value	Significant *F* values	
				5 percent	1 percent
etween replicates	3	50.11	1.17		
etween varieties	3	7,229.00	168.58	3.86	6.99
arieties × replicates (error—a)	9	42.88			
etween hours of count	2	725.39	52.87	3.40	5.61
'arieties × hours of count	6	5,854.50	426.71	2.51	3.67
emainder (error—b)	24	13.72			
Total	47				

EXPERIMENTAL WORK AND RESULTS

GERMINATION RATE OF SEED BALLS AND NAKED SEEDS

There was a marked difference among the four varieties in rate and otal germination when the germination of seed balls (containing 1 to everal true seeds) was used as the basis for comparison (table 2). In 12 hours after the seed balls were placed to germinate, variety 68 had erminated 4 percent, while variety 5194 had germinated 88 percent. This varietal difference was not due to lack of viable seed in the seed alls of variety 68, for when the seed balls were cracked open it was ound that 90 to 92 percent of them contained matured seeds. Furthermore, during preliminary seed treatments it had been found that, after thorough washing in water, from 88 to 92 percent of the seed balls of variety 68 would germinate in 192 hours.

TABLE 2.—*Comparative relationship of the rate and total germination of seed balls and naked seeds of four varieties of sugar beets*

Variety No.	Germination of seed balls after—			Germination of naked seed after—		
	42 hours	90 hours	192 hours	19 hours	42 hours	90 hours
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
68	4	30	48	22	74	94
523	6	76	78	19	64	88
550	35	89	93	23	85	97
5194	88	98	98	36	88	98
Difference required for significance:						
Odds 19:1		10.65			7.50	
Odds 99:1		13.86			9.88	

The larger difference between varieties 68 and 5194 in rate of germination disappeared when the naked seeds that had been removed from the pericarp were germinated and used as the basis for comparison (table 2). In 19 hours after the naked seeds had been placed on cotton in the Petri dishes, variety 68 had 22 percent germination, while variety 5194 had germinated to the extent of 36 percent. The results of the final counts (192 hours for seed balls as compared with 90 hours for naked seeds) are even more striking. When seed balls were used, there was a difference of 50 percent in germination percentage between varieties 68 and 5194. When naked seeds were used, this difference was reduced to 4 percent. This suggests that the main factors causing large differences between varieties in germination rate are to be found in the pericarp of the seed ball and not in the true seed.

RATE OF WATER IMBIBITION BY SEED BALLS OF THREE VARIETIES

A measure of the rate of water intake by the seed balls of three of the varieties studied affords evidence that the differences in germination are not due to variation in rate of water imbibition. Three 20-gm. lots of air-dry seed balls of varieties 68, 550, and 523 were weighed out, tied in cheesecloth bags, and submerged in distilled water to a depth of 1 inch. At the end of each soaking period a complete set was centrifuged, at a uniform rate of speed, for 2 minutes. The seed balls were then weighed, and the percentage of moisture imbibed was calculated. It will be seen from table 3 that seed balls of the slow-germinating variety 68 imbibed water as rapidly as did the other varieties. It was further observed that there was only a small increase in the rate of germination when the seed caps were pried loose from the seed balls, leaving the naked seed exposed directly to the moisture and air in the germinator but in contact with the pericarp.

TABLE 3.—*Comparison of rate of water imbibition by seed balls of three sugar-beet varieties*

Variety No.	Water intake on air-dry basis after— 2 hours	5 hours	22 hours
	Percent	*Percent*	*Percent*
68	83	100	116
550	70	84	113
523	88	94	113
Difference required for significance:			
Odds 19 : 1	7.6		
Odds 99 : 1	10.6		

TOXIC EFFECT OF WATER EXTRACTS FROM SUGAR-BEET SEED BALLS

The existence of water-soluble substances in the pericarp of sugar-beet seed balls, which affect both rate and total germination of the seed, was shown by the following experiment. Water extracts from seed balls of each of the varieties, made as described, were used in the Petri dish germinations with naked seed of variety 68. Germination started very slowly, and after about 60 hours the sprouts turned brown and died. Normal germination and growth took place

n the dishes where water had been used to moisten the substratum. The toxic effects produced by the extracts obtained from seed balls of the four varieties apparently varied inversely with the rate and total seed-ball germination (table 2); that is, the extract from the seed balls of variety 68 was most toxic to the naked seeds in the test, and the extract from 5194 was least toxic. The other two varieties, which are more or less intermediate in rate of germination of seed balls, took that position in the toxicity of their seed-ball extracts.

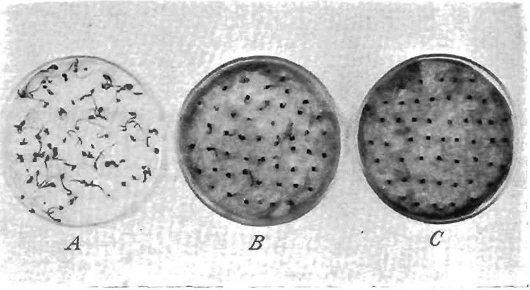

FIGURE 1.—Comparison of the toxic effect of water extracts from seed balls of two varieties of sugar beets: *A*, Check, substratum moistened with water; *B*, substratum moistened with water extract from seed balls of variety 550; *C*, substratum moistened with water extract from seed balls of variety 68. The discoloration of the cotton substratum by the seed-ball extracts is evident. Both inhibition of germination and injury to sprouts that germinated are apparent.

This is partly shown by the data in table 4. The data in the table do not, however, give a complete picture of the injury that occurs.

TABLE 4.—*Effects of water extracts from the seed balls of sugar-beet varieties on germination of naked seeds of variety 68*

Variety from which seed-ball extract was obtained	Germination of naked seeds in contact with seed-ball extracts after—		
	19 hours	42 hours	90 hours
	Percent	*Percent*	*Percent*
68	0	4	7
523	13	35	45
550	16	44	57
5194	22	50	62
Water	60	89	97
Difference required for significance:			
Odds 19 : 1		8.69	
Odds 99 : 1		11.29	

This injury is better shown by figure 1, which illustrates both the inhibition of germination and the injury to the sprouts that did germinate. Almost complete inhibition of germination is shown in figure 1, *C*, where the cotton was moistened with the water extract

from seed of 68. In figure 1, *B*, where the cotton had been moistened with an extract from seed of variety 550, more than 50 percent of the seeds germinated, but the sprouts soon turned brown and died after 60 hours.

CONCENTRATION OF TOXIC SUBSTANCES NOT CONSTANT

The amount of toxic substances present in the sugar-beet seed the varied not only with variety but also within seed lots of the same variety grown in different years or localities, and it is possibly affected by any one or all of the following factors: Climate, soil, and maturity of the seed when harvested. Extracts were made from seed of U. S. 34 that had been grown at different localities in different years. It can be seen from the data in table 5 that the toxicities of the extracts were not constant for the different seed lots of the same variety

Further tests, involving seed samples of the same variety grown the same year and in the same locality but on widely differing soil types, indicated that soil variation was an important factor influencing the amount of toxic substances in the seed ball.

TABLE 5.—*Variation in the amount of toxic substances present in the seed balls of U. S. 34 grown at different localities and in different years*

Year and locality where seed was grown from which extract was made	Germination of naked seeds after—		
	19 hours	66 hours	114 hours
	Percent	*Percent*	*Percent*
Sandy, Utah, 1932	1	14	32
St. George, Utah, 1933	2	13	31
Granger, Utah, 1934	10	44	61
Hurricane, Utah, 1935	11	51	67
Hemet, Calif., 1935	2	20	45
Logandale, Nev., 1936	0	21	40
Difference required for significance:			
Odds 19 : 1		12.31	
Odds 99 : 1		17.24	

EFFECT OF CONCENTRATION OF THE TOXIC SUBSTANCES

Decrease of rate and total germination was proportional to the concentration of the extract. When any of the extracts were diluted with water the damage to the germinating seed was decreased. The same dilution effect was also apparent when seed balls were thoroughly washed in water before the extract was taken. This reduction in the toxicity of the diluted extracts is shown in figures 2 and 3. The reduction in toxicity is evidenced by an increase in rate and total germination of the naked seed in contact with the diluted extract and also by the more normal development of the sprouts.

The toxic action of seed-ball extracts on germinating seed was not confined to the effect on seed removed from the pericarp, but was also evident when intact seed balls were used. The effect of the toxic substance already present in the seed ball can be accentuated by adding more extract so that the germination rate is further reduced, or its effect may be lessened by washing out some of the toxic substance normally present in the seed ball. In one test, seed balls of variety 550 were soaked for 24 hours in a water extract made by soaking a given weight of seed of 550 for 20 hours in five times its weight of water. The germination of this seed was then compared with that of seed

which had been washed for 24 hours in running water. The results of this test are shown in table 6.

Variety 550, which is a moderate carrier of substances having a toxic effect on germination, was greatly reduced in both rate and total germination when soaked 24 hours in a previously prepared extract from

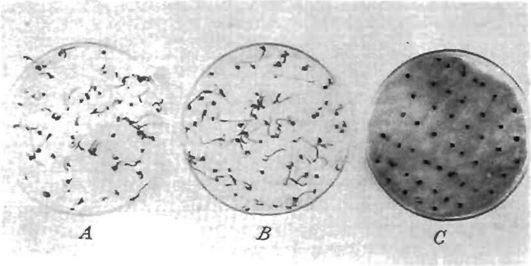

FIGURE 2.—Effect of washing the seed balls prior to obtaining the extract: *A*, Substratum moistened with water; *B*, substratum moistened with a water extract from seed balls of variety 68 that had been soaked and dried prior to making the extract; *C*, substratum moistened with a water extract from seed balls of variety 68.

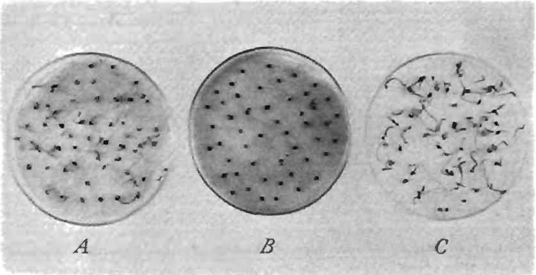

FIGURE 3.—Effect of diluting the extract after extraction from the seed balls: *A*, Substratum moistened with an extract fom seed balls of variety 68 that was diluted 2:1 with water; *B*, substratum moistened with regular extract from seed balls of variety 68; *C*, substratum moistened with water.

the same seed. The reduction in germination of variety 550 soaked in extract was not so great as the reduction in the germination of seed of variety 68, which was alternately soaked and dried three times, the same water being used for each soaking. The injury resulting to variety 68 from such treatment could not be overcome by subsequently washing the seed balls in running water. On the other hand, when seed

balls of variety 68 that had had no previous treatment were washed in running water there was a remarkable increase in both rate and total germination. It is also evident that the amount of benefit to be derived from washing seed is dependent on the amount of toxic substances carried in the seed balls of the variety and that it is unsafe to soak seed balls of a slow-germinating variety, such as 68, in a small volume of water prior to germination. Enough water to insure proper dilution should be used, and running water gives best results.

Table 6.—*Comparison of the rate of germination of seed balls of two varieties in which the amount of toxic substance had been modified by soaking or by washing*

Variety and treatment given seed balls	Germination of intact seed balls after—		
	24 hours	90 hours	192 hours
	Percent	*Percent*	*Percent*
Variety 550:			
Soaked 24 hours in extract from seed balls of 550	0	30	46
No treatment	21	43	70
Washed 24 hours in running water	41	54	81
Variety 68:			
Soaked and dried 3 times (same water)	0	2	7
Soaked and dried 3 times (water changed)	7	34	89
Washed 24 hours in running water	16	56	92
No treatment	0	13	41
Difference required for significance:			
Odds 19 : 1		13.50	
Odds 99 : 1		17.55	

COMPARISON OF SOAKING AND WASHING AS METHODS OF REMOVING TOXIC SUBSTANCES FROM SEED BALL

Presoaking of sugar-beet seed for 2 hours prior to making germination tests is recommended by the Association of Official Seed Analysts of North America (*19*). It is desirable that this recommendation be expanded to specify an adequate amount of water to insure removal of toxic substances from the seed ball. Experiments showed that variation in the quantity of water in which seed was soaked or failure to wash and properly dry samples after soaking caused variations in the results.

Table 7 gives the data obtained from an experiment to test the effect of presoaking sugar-beet seed in different quantities of water and the further effect of washing or not washing the seed balls after the presoaking period. Seed of two varieties was used, 68 and 550. The seed of 68 is a heavier carrier of the toxic substances; the seed of 550 carries only moderate amounts. The F values (*17*) in table 7 indicate that there is a real difference between the behavior of these two varieties. The F values show further that varying the quantity of water in which the seeds were soaked had a marked effect on subsequent germination. Washing the samples for 5 minutes in running water after the soaking period also had a marked effect, and it will be further seen that the 5-minute wash period after soaking was of much more importance when small quantities of water were used for presoaking.

Further comparison between the effectiveness of soaking and washing the seed balls for different lengths of time prior to germination is given in table 8. It is evident that washing in running water was more effective than soaking for the removal of toxic substances and that washing up to 6 hours was beneficial. Washing beyond 6 hours was of no apparent advantage.

TABLE 7.—*Comparison of the effect of different quantities of water used to presoak sugar-beet seed, and the further effect of washing the samples in running water for 5 minutes after the 22-hour soaking period*

GERMINATION DATA

Treatment of seed balls prior to placing them in Petri dishes to germinate: Volume of water used to soak seed 22 hours	Treatment following soaking	Variety No. 68: 42 hours	Variety No. 68: 114 hours	Variety No. 68: 218 hours	Variety No. 550: 42 hours	Variety No. 550: 114 hours	Variety No. 550: 218 hours
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
3 times weight of seed	Washed 5 minutes	12	48	60	16	44	58
	Not washed	1	20	28	11	36	47
6 times weight of seed	Washed 5 minutes	15	48	61	29	51	69
	Not washed	3	39	54	15	38	46
9 times weight of seed	Washed 5 minutes	14	46	64	33	62	69
	Not washed	9	41	61	27	49	66
16 times weight of seed	Washed 5 minutes	23	54	70	37	66	75
	Not washed	12	51	68	35	55	67
Washed 22 hours in running water.		18	73	80	38	63	76
No treatment given seed.		0	12	45	1	37	63
Difference required for significance: Odds 19 : 1		12.0					
Odds 99 : 1		15.6					

COMPARISON OF CALCULATED *F* VALUES WITH *F* VALUES FOR THE 5- AND 1-PERCENT POINTS

Source of variation	Calculated *F* value	Significant *F* values: 5 percent	Significant *F* values: 1 percent
Between varieties	63.73	3.94	6.90
Washed and not washed	114.69	3.94	6.90
Between quantities of water used for soaking	48.47	2.70	3.98
Washing or not washing × different quantities of water	13.10	2.70	3.98
Variety × washing or not washing × quantity of water	15.10	2.70	3.98

TABLE 8.—*Comparison of the effect of soaking seed in 5 times its weight of water and washing seed in water for varying periods of time*

[Test with variety 68]

GERMINATION DATA

Treatment	Germination of intact seed balls after — 4 days	6 days	8 days
	Percent	*Percent*	*Percent*
No treatment	8	29	50
Soaked 3 hours	17	35	65
Washed 3 hours	16	39	78
Soaked 6 hours	23	47	75
Washed 6 hours	41	76	88
Soaked 24 hours	15	41	74
Washed 24 hours	38	79	87
Difference required for significance: Odds 19 : 1		11.5	
Odds 99 : 1		14.82	

TABLE 8.—*Comparison of the effect of soaking seed in 5 times its weight of water, and washing seed in water for varying periods of time*—Continued

COMPARISON OF THE CALCULATED *F* VALUES FOR THE 8-DAY COUNT WITH *F* VALUES FOR THE 5- AND 1-PERCENT POINTS

Source of variation	Calculated *F* value	Significant *F* values	
		5 percent	1 percent
Between replications	3.45	5.59	12.25
Between hours of treatment	13.74	4.74	9.55
Between soaking and washing	10.32	5.59	12.25
Soaking or washing × hours of treatment	2.45	4.74	9.55

It seems rather important that where presoaking is made a part of laboratory procedure, it should be done in standard containers of sufficient size to allow for an adequate volume of water; otherwise

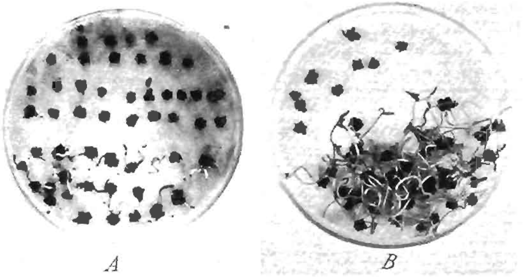

FIGURE 4.—Comparison of the condition of sprouts from seed balls not washed and those washed prior to germination in Petri dishes. *A*, Unwashed seed balls of variety 550, 1 week after they had been placed in a Petri dish on cotton moistened with water. *Damage to the sprouts by the substances that arise* from the seed ball is very apparent. *B*, Seed balls of variety 550 that were washed 22 hours in running water before being placed on moistened cotton in a Petri dish. The healthy appearance of the sprouts is apparent even after 2 weeks of growth.

the seed should be washed in running water. The appearance of the sprouts in blotter germination tests readily indicates whether the seed balls are being properly presoaked or washed. If the tips of the sprouts become darkened during the test, this is evidence that toxic substances have not been properly removed from the seed balls. Figure 4 shows the condition of sprouts from seed balls that had been thoroughly washed and from seed balls that had not been washed prior to being placed in Petri dishes to germinate. Possibly it should be pointed out here that when sugar-beet seed is germinated on cotton in Petri dishes it is somewhat more sensitive to the presence of toxic substances than when it is germinated on blotting paper. However, practical experience in several seed laboratories has shown this same relationship to apply to blotter tests.

DRYING SEED BALLS AFTER SOAKING OR WASHING

The importance of freeing the seed balls of all excess moisture, after washing or soaking and before placing them in a moist chamber to germinate, was demonstrated on several occasions. Figure 5 shows a comparison between the percentage germination of seed balls placed on moist cotton immediately after washing and the percentage germination of seed balls that were air-dried after the washing process.

It seems advisable that some standard procedure should be adopted that will insure removal of the excess moisture that might interfere with respiratory processes of the germinating seed. Practical ex-

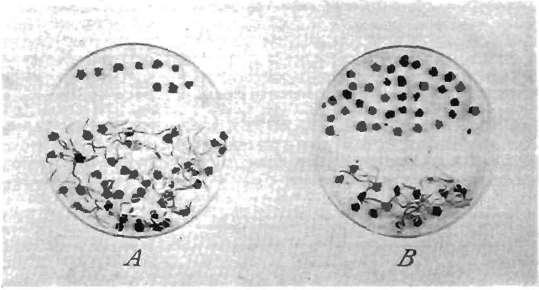

FIGURE 5.—Cultures showing the importance of properly drying the seed balls after the presoaking period and before placing them in a moist chamber to germinate. *A*, Seed balls air-dried after washing, before being placed on cotton. After 5 days, 82 percent showed visible sprouts. *B*, Seed balls placed on cotton immediately after washing. After 15 days only 28 percent had germinated.

perience of several seed laboratories seems to indicate that thorough blotting removes sufficient surface moisture to prevent the difficulties as outlined above.

DISCUSSION

The finding that water-soluble subtances toxic to germinating seeds exist in the pericarp tissue of sugar-beet seed balls and the demonstration that these can be removed by proper washing afford an explanation for the improved germination that has been found to result from washing or soaking sugar-beet seed. In regard to the so-called "stimulating effects" from liquid seed treatments with inorganic salts or fungicides, the possibility that the effect has been brought about by removal of toxic substances should be taken into consideration. In some experiments by C. H. Smith, of the Salt Lake City laboratory, as well as in the early experiments of Stehlík and Neuwirth (*18*), it was found that water alone was as effective in increasing germination as solutions of various inorganic salts. Hanley and Woodman (*7*) and Garner and Sanders (*6*) report that treatment of sugar-beet seed balls with sulfuric acid leads to an increase in both rate of germination and total germination. They attribute the increase in germination to a greater permeability of the hard seed balls, which allows the processes connected with germination to take place

more rapidly. Stehlík and Neuwirth (*18*) give data to show that this effect is no greater than that obtained by soaking in water. It is obvious that decortication by sulfuric acid removes some of the corky material of the seed ball and with it some of the toxic substances. This treatment is also followed by thorough washing, which, in itself, would remove some of the toxic materials. Inasmuch as both washing in water and soaking in sulfuric acid result in the removal of toxic substances from the seed ball, it is to be expected that both treatments would result in increased germination.

The substances in sugar-beet seed balls that produce the toxic effect on germination are probably accumulated in the pericarp during seed development. It is possible that chemical changes in these substances take place during seed germination; however, since the toxic effect is produced by substances located in the pericarp rather than in the true seed, the primary source of these substances does not appear to be germination byproducts as has been reported in lettuce seed by Shuck (*16*).

The fact that seed from different varieties and different seed lots of the same variety may vary widely in the amount of toxic substances present makes it difficult to give a generalized statement regarding the benefits to be derived from presoaking. The amount of benefit to be derived from presoaking, the length of the presoaking period, and the advantage of washing as compared with soaking will all depend on the amount of toxic substances present in the lot of seed used and also on the substratum on which the tests are made.

Varietal differences in germination due to the presence of toxic substances are most clearly shown on the cotton in Petri dishes. When blotters, sand, or soil are used as the germinating media, there is a gradual decrease in the toxic effect proportional to the ability of the substratum to remove substances from the seed ball. This is similar to the finding of Borriss (*2*), who concluded on the basis of tests with *Vaccaria pyramidata* that the germination-promoting effect of the soil was not due to the presence of any stimulative factor, but rather to the removal of an inhibitory substance from the seeds by the absorptive power of the soil complex. Experiments by the authors indicate that in the field the effect of toxic substances is so reduced that, although the effect on rate of germination may be noticeable, little effect on total germination and no effect on yield have been apparent. Since the purpose of laboratory tests is to forecast the field performance of seed, it is desirable to guard against interference from toxic substances. Inasmuch as they do sometimes interfere in blotter tests, it seems advisable to include their removal in the germination procedure. Proper washing and drying of the seed balls prior to placing them in the blotters will increase the rate of germination, avoid damage to the sprouts, and reduce the variation between samples.

SUMMARY

Seed balls from different varieties of sugar beets varied widely in rate and total germination even though an equivalent number of seed balls from each variety was known to contain mature seed. These differences between varieties were largely dissipated when the naked seeds, removed from the seed ball, were germinated or when seed balls from the different varieties were thoroughly washed prior to the germination test.

The amount of water-soluble substances present in sugar-beet seed balls varied not only with variety but also within seed lots of the same variety grown in different years or localities, and is possibly affected by any one or all of the following factors: Climate, soil, and maturity of the seed when harvested.

Water-soluble substances present in the seed ball were found to produce a toxic effect on germinating sugar-beet seed, both retarding germination and killing the radicles. This effect may be a very important factor among lots of sugar-beet seed in causing the differences observed in rate and total germination of seed lots in seed laboratories.

The adequate removal of these substances from the seed ball seems to be advisable in laboratory procedure where blotters or similar substrata are being used for germination tests. Inasmuch as the amount of toxic substances varies with variety and within different seed lots of the same variety, it seems advisable to recommend a laboratory procedure that will insure their removal from all seed samples.

The water-soluble toxic substances of sugar-beet seed balls can be removed by either soaking or washing in running water, a 6-hour period of treatment being adequate for their removal from seed balls carrying large amounts of the toxic substances. Soaking the seed in a sufficient volume of water to insure dilution of toxic substances was definitely beneficial, but not so effective as washing the seed in running water.

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STEMPHYLIUM LEAF SPOT OF RED CLOVER AND ALFALFA [1]

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INTRODUCTION

One of the foliage diseases of red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.) is caused by a fungus formerly known as a *Macrosporium*, but more recently as a species of *Stemphylium*. The causal fungus, which is characterized by echinulate conidia and has an ascigerous stage belonging in the genus *Pleospora*, has been known previously as a parasite of red clover and alfalfa, but unfortunately has sometimes been confused with *Macrosporium sarcinaeforme* Cav., a fungus that has smooth-walled conidia, has no known ascigerous stage, and is known to occur only on red clover in nature. The investigation reported in this paper was designed to trace the life history of the echinulate-spored fungus and to clarify any confusion that may exist in the literature regarding its identity and relationship to *M. sarcinaeforme* and other similar fungi on red clover and alfalfa.

Krakover (*9*)[2] and Horsfall (*6*) have shown that the fungus on red clover has smooth-walled conidia and corresponds very well with Cavara's original description of that species. Wiltshire (*24*) has transferred this fungus to the genus *Stemphylium*, and according to him, it should be known as *S. sarcinaeforme* (Cav.) Wiltshire. Gentner (*5*) reported the echinulate-spored fungus to be the cause of a disease of both red clover and alfalfa in Germany, but he misidentified it as *Macrosporium sarcinaeforme*. He also reported perithecia of *Pleospora herbarum* (Pers. ex Fr.) Rabh. in pure cultures of this echinulate-spored fungus. The association of this ascomycete with a fungus having sarcinaeform, warty conidia was shown by the Tulasne brothers (*21*) in 1863 and has since been confirmed by several other investigators. This work has been reviewed by Wiltshire (*24*). In New York, Horsfall (*6*) obtained single-ascospore cultures of what he thought was *P. herbarum* from overwintered red clover stems two seasons in succession. He reported that these cultures were identical in every respect with those of the *Macrosporium* from lesions on alfalfa leaves, including the production of spiny conidia. He suggested, therefore, that the *Macrosporium* from alfalfa and its possible sexual stage, *P. herbarum*, may occur on both red clover and alfalfa, but that *M. sarcinaeforme* from red clover is limited to that suscept in nature. In the present paper the spiny-spored fungus is reported to be pathogenic on both alfalfa and red clover and is identified as *Stemphylium botryosum* Wallr.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 845.

The ascigerous stage has been found on dead red clover stems and has developed on sweetclover stems and on potato-dextrose agar. It is identified as *Pleospora herbarum*. *P. denotata* (Cke. and Ell.) Sacc., originally described from dead stems of red clover and differing from *P. herbarum* only in the larger size of the ascospores, is considered by the writer to be a synonym of *P. herbarum*.

THE DISEASE

On red clover the disease occurs mainly on leaf tissue but it may also occur on stems and petioles. Leaf lesions are irregular in shape and dark brown to black in color (fig. 1, *A* and *B*). Around each

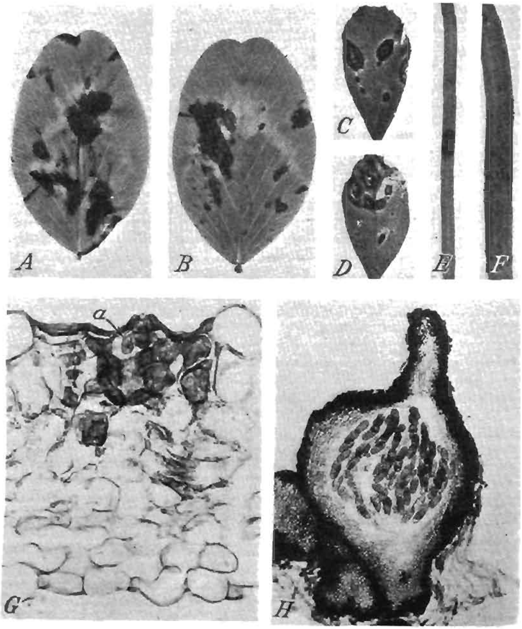

FIGURE 1.—*A* and *B*, Leaves of red clover artificially inoculated with crushed perithecia of *Pleospora herbarum*, about natural size; *C* and *D*, natural infection of alfalfa leaves by *P. herbarum*, about natural size; *E* and *F*, petiole and stem of red clover artificially inoculated with crushed perithecia of *P. herbarum*, × about 1½; *G*, early stage of infection of red clover leaf by *P. herbarum* showing enlarged mycelium (*a*) in substomatal cavity, ×about 430; *H*, cross section of a perithecium of *P. herbarum* grown on a sweetclover stem, ×about 100.

esion a straw-colored halo often develops and maintains itself in advance of the enlarging lesion. Under favorable conditions the esions increase in size quite rapidly and coalesce until much of the leaf s involved. Under field conditions the disease is difficult to diagnose n stems and petioles, as it is often associated with the black stem disease caused by *Phoma trifolii* Johnson and Valleau (*8*). However, small black linear lesions are produced on stems and petioles following noculation with pure cultures of the causal organism (fig. 1, *E* and *F*). On stems these lesions have never developed extensively, and it appears unlikely that such infections are capable of doing appreciable damage o the plant. On petioles, however, the lesions sometimes develop ufficiently to cause breaking at the locus of infection.

PATHOLOGICAL HISTOLOGY

A modification of the cleared-leaf method described by Peace (*13*) was used for observing spore germination and early stages of infection n leaf tissue. Bits of leaf tissue were cleared in alcohol-acetic acid 0–50, stained with cotton blue in lactophenol, then mounted in lear lactophenol. For more detailed observation on early stages of nfection and host-parasite relations, stained microtome sections were nade of infected leaf tissue. The tissue was fixed in Karpechenko's nodification of Navaschin's fixing fluid, consisting of equal parts of olution A (195 cc. water, 30 cc. glacial acetic acid, and 3 gm. chromic acid) and solution B (195 cc. water, 30 cc. formalin (37 percent ormaldehyde)) mixed just before using. The material was dehydrated and infiltrated with paraffin, according to the schedule given by Rawlins (*16*), by the use of cedar oil following a graded series of alcohols up to 95 percent. Sections were cut 10μ and 15μ in thickness. Staining with safranin and fast green gave satisfactory differentiation between host and parasite.

Since spore germination and penetration of the mycelium into the eaf are similar for both ascospores and conidia, the following description of these processes applies to both. Illustrations were drawn only or ascospores. Spores germinate from several of the cells that comprise the spore (fig 2, *A*). Penetration into the leaf is usually through tomata but may be directly between cells. Usually a strand of nycelium grows over the leaf surface until it reaches a stoma, becomes omewhat swollen over the opening, and an infection hypha enters he substomatal cavity (fig. 2, *B*). Cases have been observed, however, where hyphae have grown directly across stomata without entering. In the substomatal cavity the fungus usually forms two or three short, thick strands of mycelium, about two or three times as thick as aerial mycelium or mycelium subsequently formed within the host tissue (fig. 2, *B*). This bulbous mycelium apparently makes very little growth until several host cells immediately around the stoma have been killed (fig. 1, *G*); it then continues to grow intercellularly through the leaf tissue as fine strands of mycelium (fig. 2, *D*).

In the few cases of direct penetration that were observed, a germ ube, about twice the thickness of other germ tubes, had grown only a short distance from the spore and had then penetrated the leaf between epidermal cells (fig. 2, *C*). After gaining entrance into the eaf the fungus grows intercellularly through the leaf, killing the host cells as it progresses. During the early stages of infection, very few

strands of mycelium were observed inside the leaf in either microtome-sectioned material or in cleared leaves stained with cotton blue. Conidiophores and conidia were produced on leaf lesions in about 5 to 8 days after inoculation (fig. 2, *E*).

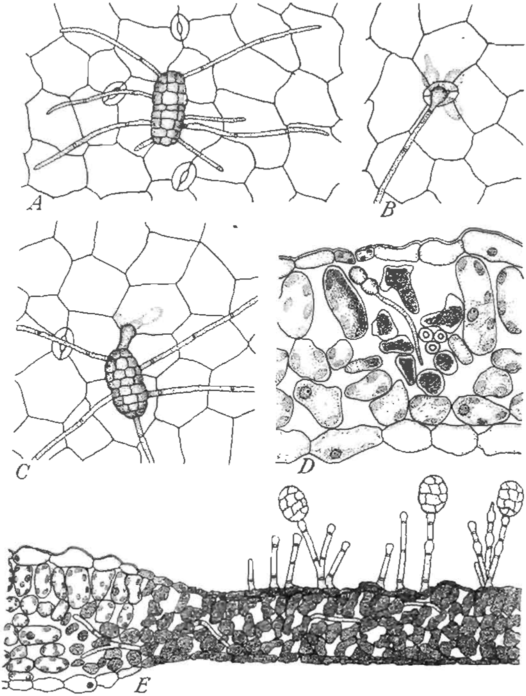

FIGURE 2.—Camera-lucida sketches of *Pleospora herbarum* on red clover leaf tissue: *A*, Germinating ascospore, × about 420; *B*, early stage of stomatal penetration showing enlarged hyphae in substomatal cavity, × about 420; *C*, early stage of direct penetration, × about 420; *D*, early stage of infection, showing enlarged mycelium in substomatal cavity and normal-sized mycelium in region of mesophyll and spongy parenchyma, × about 420; *E*, leaf lesion showing conidiophores and conidia, × about 275.

PATHOGENICITY OF THE FUNGUS

The organism is readily isolated from field material by placing small pieces of surface-sterilized diseased leaf tissue on acidified potato-dextrose agar. From characteristic leaf lesions pure cultures of the organism are obtained in nearly every case. Although isolates of the organism differ slightly in color they are usually white during the first few days of growth, after which the mycelium near the center of the colony develops dark pigments that appear green from the top and dark brown from the under side of the culture. Perithecial primordia begin to appear in the cultures in about 5 to 8 days. It is quite common to find white tufts of mycelium in some cultures, and in a few instances sterile white sectors have occurred similar to those observed by Ellis (*4*) in cultures of *Pleospora herbarum* from *Euphorbia*. While zonation was observed in culture, no attempt was made to determine its cause. Ellis (*4*) attributed the zonation in cultures of *P. herbarum* from *Euphorbia* to temperature variations.

The pathogenicity of the organism was proved by inoculating plants grown in the greenhouse and reisolating the fungus from the lesions produced. Infection was obtained both by spraying the plants with a suspension of conidia obtained from diseased leaves and by smearing a suspension of ascospores and perithecial fragments on the plant with a camel's-hair brush. After all inoculations the plants were kept in a glass-enclosed chamber at a high atmospheric humidity for 2 to 5 days before being removed to a greenhouse bench. Lesions on leaf tissue developed in about 3 to 4 days, whereas stem and petiole lesions were not in evidence until about 10 to 14 days after inoculation.

It was beyond the scope of this investigation to determine the complete host range of this fungus. Inoculations were made, however, on a number of plants during the winter of 1937 with a culture of the organism isolated from diseased red clover leaves collected at Marshfield, Wis. No infection was obtained on the following plants: *Melilotus alba* Desr., *M. officinalis* Lam., *Trifolium incarnatum* L., *T. involucratum* L., *T. agrarium* L., and *T. procumbens* L. Slight infection was produced on *Medicago arabica* Huds., and *T. resupinatum* L. *Medicago sativa* proved to be moderately susceptible. In 1938, inoculations were made on *Melilotus alba* and *Medicago sativa* with a culture of the fungus obtained from diseased leaves of *T. pratense* near Madison, Wis. The organism produced small lesions on *Melilotus alba* and on *Medicago sativa*. Other plants were not available for inoculation at that time. From these limited trials it appeared that different isolates of the fungus differ slightly in pathogenicity, but the organism isolated from *T. pratense* is primarily a pathogen of that plant.

The organism infects the seeds of several leguminous plants. Gentner (*5*) reported it on seeds of red clover, white clover, alsike clover, crimson clover, and lucerne. From F. R. Jones [3] the writer obtained a culture of this fungus isolated by him from living seeds of *Melilotus alba*. Tested by inoculation, it proved to be pathogenic on *M. alba* and *Trifolium pratense*. Thus *M. alba* can now be added to the list of plants that may carry this pathogen in or on the seeds.

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[3] Senior pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

MORPHOLOGY OF THE FUNGUS

ASCIGEROUS STAGE

The perfect stage of the fungus has been found on dead stems of red clover and has developed in culture on potato-dextrose agar and on sweetclover stems. It is here identified as *Pleospora herbarum*. Perithecia are typically globoid and sometimes possess a slender neck. The latter character is very pronounced on perithecia produced on sweetclover stems but is usually lacking on perithecia produced on potato-dextrose agar. Each fruiting body is usually distinct, has a black membranous wall, and produces many asci (fig. 1, *H*). When the fungus is grown on either potato-dextrose agar or sweetclover stems, perithecia are formed in about 10 to 14 days but asci and ascospores are not produced until about 4 or 5 months later. A temperature of 10° to 12° C. is most favorable for ascospore production. Ellis (*4*) reported that cultures of *P. herbarum* from *Euphorbia* develop perithecia with fully developed asci in about a month, and added (*4, p. 111*): "It is probable that any check to the vegetative growth of the mycelium will be followed by the production of increased numbers of reproductive structures, though all these may not necessarily mature."

Asci are elongate, cylindrical, and typically eight-spored. They measure 183μ to 267μ by 27μ to 37μ, with a mean length of $39 \pm 0.2\mu$ and a mean diameter of $34 \pm 0.3\mu$.

Ascospores are muriform and yellowish to brown in color. They are rounded at both ends, have seven cross septa and three to five longitudinal septa. They measure 32μ to 48μ by 14μ to 21μ, with a mean length of $39 \pm 0.4\mu$ and a mean diameter of $17 \pm 0.2\mu$. The standard error was calculated from the formula

$$S_x = \sqrt{\frac{\Sigma(x^2) - \frac{(\Sigma x)^2}{n}}{n(n-1)}}$$

Each calculation was based on 100 random measurements.

The method of ascospore discharge is essentially like that described by other investigators (*1, 15, 18*) and will be only briefly reviewed here. Asci are readily liberated from the perithecium by placing the latter on a glass slide in a drop of water, covering it with a cover slip, and applying light pressure. The perithecium ruptures usually at one side of the bulbous portion, and the asci pass out into the surrounding water. The ascus wall consists of two layers, an outer one that is thin and quite firm and an inner one that is thicker and elastic. The two walls are not visibly distinguishable, however, until the outer wall is ruptured during the process of spore discharge.

When an ascus is liberated from the perithecium, it imbibes water and begins to swell (fig. 3, *A*). The outer wall, being inelastic, bursts, usually at the apical end, and contracts slightly, forming several wrinkles or folds around the inner ascus wall (fig. 3, *B*). Simultaneously the inner membrane stretches rapidly and the ascus becomes nearly twice its original length with little or no increase in diameter (fig. 3, *B*).

Asci may elongate immediately on coming in contact with water or several minutes thereafter, but the actual process of elongation requires only 10 to 20 seconds. Immediately after the outer ascus wall bursts and during the elongation of the inner wall, the spores and ascus contents move to the upper portion of the ascus. Usually all the spores except one move above the ring formed by the outer wall. Spores that remain in the lower portion of the ascus are not expelled at the time of spore discharge.

A stretched ascus may discharge its spores immediately or several minutes after it has elongated, or it may elongate and not discharge its

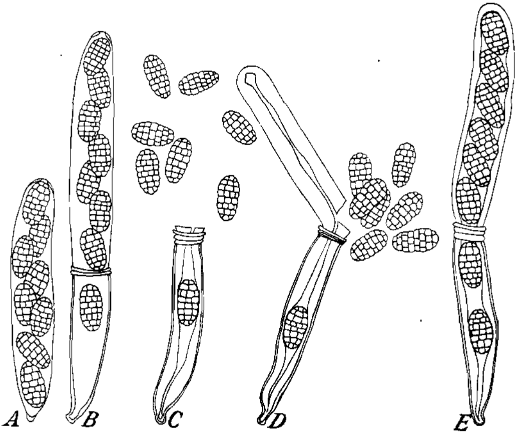

FIGURE 3.—Camera-lucida sketches of ascospore discharge in *Pleospora herbarum*: *A*, Ascus as it appears when first forced from perithecium; *B*, same ascus as *A* after outer wall has burst and inner wall has elongated; *C* and *D*, asci which have discharged their spores; *E*, ascus from which spores were not discharged. Note how the inner wall has become swollen in *C, D*, and *E*. × about 240.

spores (fig. 3, *E*). The stretched ascus usually bursts just above the ring formed by the outer ascus wall and the spores are expelled into the surrounding medium. In some cases an ascus bursts on one side and the spores pass out through the rift thus formed (fig. 3, *D*), while in other cases the entire upper portion of the stretched ascus is torn off and the spores are scattered in any direction (fig. 3, *C*). Spores are discharged so quickly that their exit from the ascus cannot be followed with the eye; from observations it appears that they are discharged with considerable force, but because of the resistance offered by the water they remain near the point of release. The inner wall of the ascus thickens considerably soon after stretching and asci that discharge their spores do so before the thickening occurs. Spores that

are not discharged germinate while inside the ascus and the germ tubes grow out through the thickened wall.

CONIDIAL STAGE

The conidial stage of the fungus is considered to be *Stemphylium botryosum* Wallr. (*22*), the species upon which Wallroth founded this genus in 1833. Conidia are rarely produced on potato-dextrose agar, but under conditions of high humidity numerous conidiophores and conidia are produced on leaf lesions. The conidiophores are brown, upright, stout-appearing structures that are either single or grouped in fascicles rarely composed of more than four hyphae. They are slightly bulbous at their bases and also at the distal end where the conidia are borne. They measure 3.5μ to 5.5μ in width but differ considerably in length, depending on environmental conditions and the number of conidia they produce. After a conidium has been produced, the end of the conidiophore grows out, producing new cells and a new conidium. As a result of the continued apical growth, a conidiophore may grow to considerable length and have a nodulose appearance, the swollen cells of the conidiophore indicating the points at which conidia were borne (fig. 2, *E*). Conidia that are not dislodged from the ends of the conidiophore are pushed to one side by the new growth and appear to be borne laterally on the conidiophore.

The conidia are olive brown muriform, and echinulate. They are oblong in shape and often constricted at the center by a median cross septum. They measure 24μ to 40μ by 14μ to 23μ, with a mean length of $31 \pm 0.4\mu$ and a mean width of $19 \pm 0.2\mu$.

There can be no doubt that the perithecial and conidial stages described here belong to the same fungus. On leaf lesions resulting from inoculations with ascospores the fungus produces the echinulate conidia, and cultures from these conidia produce the perithecial stage when grown on sweetclover stems or on potato-dextrose agar. On potato-dextrose agar, cultures started from conidia and ascospores are similar in appearance. On sweetclover stems a few conidia are produced in addition to the perithecia.

The fungus is homothallic. Perithecia and ascospores have been produced on sweetclover stems from either single-ascospore or single-conidium cultures.

TAXONOMY

The fact that fungi similar to the one described above on red clover have been described on several different host plants raises the question whether the red clover organism is identical with one or more of those previously described. Many of the earlier descriptions were of only the conidial or ascigerous stage of the fungus, since no connection was established between the two spore stages. Where possible, cultures of the organisms from the various hosts were obtained and when this material was lacking, herbarium specimens and written descriptions were used. The following described species have been considered: *Pleospora denotata* (Cke. and Ell.) Sacc.; *P. lycopersici* É. and É. March.; *Macrosporium medicaginis* Cugini; *M. sarcinaeforme* Cav. as discussed by Malkoff (*10*), Gentner (*5*), and Hülsenberg (*7*); *M. meliloti* Pk.; and *Thyrospora sarcinaeforme* (Cav.) Tehon and Daniels. A summary of the information obtained regarding most of these fungi is given in table 1.

TABLE 1.—*Summary of information on fungi considered in relation to Stemphylium botryosum*

Fungus	Described or discussed by—	Host	Spore measurements	Character of conidium wall	Ascigerous stage
Macrosporium meliloti Pk.[1]	Peck (*14*)	*Melilotus alba* Desr.	µ 25–50 long	Smooth	
Macrosporium sarcinaeforme Cav.[1]	Cavara (*2*)	*Trifolium pratense* L.	24–28×12–18	do	
Macrosporium sarcinaeforme Cav.[2]	Malkoff (*10*)	*Trifolium pratense*.	25.2–33.6×16.8–22.4	Echinulate	
Macrosporium medicaginis Cugini.[1]	Traverso (*20*)	*Medicago sativa* L.	25–35×16–18	Smooth	
Macrosporium sarcinaeforme Cav.[2]	Gentner (*5*)	*Medicago sativa* *Trifolium pratense*.	17–40×8.7–29	Echinulate	*Pleospora herbarum* (Pers. ex Fr.) Rabh.
Thyrospora sarcinaeforme (Cav.) Tehon and Daniels.[2]	Tehon and Daniels (*19*).	*Medicago sativa*.		do	
Macrosporium sarcinaeforme Cav.[2]	Hülsenberg (*7*).	*Medicago sativa*.		do	
Stemphylium botryosum Wallr.	Smith	*Trifolium pratense*.	24–40×14–23	do	*Pleospora herbarum*.
		Medicago sativa	24–38×14–22	do	*Pleospora herbarum* (*P. denotata* (Cke. and Ell.) Sacc.)

[1] Considered distinct from *Stemphylium botryosum*.
[2] Considered synonymous with *Stemphylium botryosum*.

As will be shown later, most of these organisms are morphologically identical with *Pleospora herbarum* or its conidial stage, *Stemphylium botryosum*, and it appears that, if we are to accept morphological differences rather than small differences in pathogenicity as a basis for making specific differences, then *P. herbarum* and its conidial stage, *S. botryosum*, consist of a group of fungi morphologically alike but possessing some degree of physiological specialization. Wiltshire (*24*) has recently shown that a number of fungi previously described and named in their conidial stage as distinct species are in reality synonymous with *S. botryosum*. He also states that the association of this conidial type with *P. herbarum* as its ascigerous stage was known as early as 1863 by the Tulasne brothers (*21*).

The ascospores of *Pleospora herbarum* from red clover are a little longer than the measurements given by Saccardo (*17*) for that species. This fact gives some support to the possibility of considering the organism from red clover as *P. denotata*, a fungus which was described on dead stems of red clover and is separated from *P. herbarum* by the larger size of its ascospores. However, measurements of most of the spores of the red clover fungus come within the limits given for *P. herbarum* and it is considered best, for the present at least, to consider it as the latter species. Little doubt exists, however, that the organism described by Cooke and Ellis is the same as the one described here, but it is doubtful whether *P. denotata* can be considered as a species distinct from *P. herbarum*. Type material of *P. denotata* is very limited, but in the small portion examined the ascospores were found to be shorter than the length given in the original description of that fungus and most of them were within the limits given for *P. herbarum*. Many of the ascospores appeared as normal as ascospores from freshly collected field material, and it is assumed therefore that they had not become much shrunken. It seems better to extend the

limits of spore size of *P. herbarum* sufficiently to include the organism under consideration here rather than try to distinguish it as a distinct species on the difference of a few microns in spore length, particularly since the ascigerous and conidial stages correspond so closely to *P. herbarum* and its conidial stage, *Stemphylium botryosum*, respectively. Furthermore, it has been observed during these studies that environment has some effect on the size of ascospores of this fungus. Ascospores from perithecia on dead stems of red clover collected in the field measured 32μ to 48μ by 14μ to 21μ, whereas ascospores of the same fungus produced in culture on sweetclover stems measured 32μ to 54μ by 12μ to 22μ. However, the average size of the spores from red clover stems and sweetclover stems was 39.7μ by 17μ and 40.6μ by 16.8μ, respectively, from which it is apparent that the larger spores produced on sweetclover stems were comparatively few. This difference shows, however, that culture media and environmental conditions influence the size of ascospores to some degree.

From diseased leaves of alfalfa the writer isolated an echinulate-spored fungus that is considered to be *Stemphylium botryosum*. The pathogenicity of the fungus has been proved by inoculating alfalfa plants and again reisolating the fungus from the lesions produced. Conidia are rarely produced in culture, but abundant conidiophores and conidia are produced on leaf lesions. The conidiophores are sometimes branched, have the characteristic enlarged tips, and bear sarcinaeform spores that measure 24μ to 38μ by 14μ to 22μ. On potato-dextrose agar, the mycelium is white during the early stages of growth but later develops dark pigments that appear green from the top of the culture and dark brown or black from the bottom. On sweetclover stems or potato-dextrose agar, cultures of the organism produced *Pleospora herbarum* as the ascigerous stage. The ascigerous stage has not been observed on alfalfa stems collected in the field, but ascospores produced on sweetclover stems measured 32μ to 46μ by 13μ to 21μ.

Under field conditions alfalfa leaf lesions appear as dark-brown sunken areas that are often surrounded by a straw-colored halo (fig. 1, *C* and *D*). Concentric zones sometimes develop in old lesions.

Inoculation experiments indicate, however, that physiologic races of *Pleospora herbarum* exist on red clover and alfalfa. A suspension of conidia obtained from diseased alfalfa leaves was used to inoculate red clover and alfalfa plants. Leaves on alfalfa plants were completely killed in about 5 to 8 days, whereas no macroscopically visible infections appeared on red clover until 16 days after inoculation and then infection was very slight. When the two hosts were inoculated with a suspension of conidia from red clover, leaves of the latter host were killed in about 5 to 7 days after inoculation, whereas only small lesions were produced on alfalfa 14 days after inoculation. Similar inoculations were made with a suspension of ascospores and perithecial fragments from crushed perithecia, and in each case the organism collected from red clover was much more virulent on red clover than on alfalfa, while the organism collected from alfalfa was always more virulent on alfalfa than on red clover; in fact, the alfalfa organism rarely produced infection on red clover.

That the two organisms are different physiologic races is also supported by field observations. During the two seasons the red clover organism has been under observation the disease has appeared during

the early part of June, whereas in the season of 1938 the disease on alfalfa appeared in August and September. In June 1938, the disease was quite prevalent on red clover plants adjacent to a plot of alfalfa to which there was ample opportunity for the organism to spread. Several attempts were made to find it on the alfalfa but without success. While these data are not extensive enough to be conclusive, they do give evidence of some degree of physiological specialization on the two hosts.

Tehon and Daniels (*19*) described a brown leaf spot of alfalfa caused by an echinulate-spored fungus which they considered identical with *Macrosporium sarcinaeforme* Cav. Following Elliott's (*3*) suggestion that *M. sarcinaeforme* is not a good member of the genus *Macrosporium*, and assuming the forms on red clover and alfalfa to be identical, they erected a new genus, *Thyrospora*, with *T. sarcinaeforme* (Cav.) Tehon and Daniels, as the type species. It has already been shown by other investigators (*6, 24*) and confirmed by the writer's own observations that Tehon and Daniels were incorrect in identifying their organism as *M. sarcinaeforme*, as the spores of the organism they described are echinulate, whereas spores of the red clover organism described by Cavara are smooth.

The organism described by Tehon and Daniels is here considered morphologically identical with *Stemphylium botryosum* described above on alfalfa. Spores and conidiophores obtained from diseased alfalfa specimens received from L. R. Tehon (fig. 4, *C*) correspond very well with spores and conidiophores of *S. botryosum* collected from diseased alfalfa by the writer (fig. 4, *B*). Wiltshire has already shown, moreover, that the genus *Thyrospora*, erected by Tehon and Daniels for this fungus, is identical with the genus *Stemphylium*, and he has accordingly transferred members of the genus *Thyrospora* to the genus *Stemphylium*.

Macrosporium medicaginis Cugini (*20*) apparently causes a disease of alfalfa similar to that caused by *Stemphylium botryosum*. It was hoped that a specimen of Cugini's collection could be obtained in order that the fungus described by him might be compared with *S. botryosum*. Since the organism was collected and described in Italy, an attempt was made to locate a specimen[4] at several stations in Italy, including the Experimental Agrarian Station at Modena, where Cugini worked. Since none was found it appears unlikely that any of the original material of *M. medicaginis* Cugini exists. Illustrations of leaf lesions given by Traverso (*20*) appear to have been drawn from lesions similar to those produced on alfalfa by *S. botroysum*. Spore measurements of *M. medicaginis* and *S. botryosum* are quite similar, but spore shape of *M. medicaginis*, as illustrated in Traverso's article, does not agree well with spore shape of *S. botryosum*. Furthermore the spores of *S. botryosum* are echinulate, whereas those of *M. medicaginis* are described as smooth. If this description is correct, then *M. medicaginis* is distinct from *S. botryosum*. In fact, the description given for *M. medicaginis* is not completely consistent with that of any fungus subsequently described on *Medicago sativa*. The fact that *Macrosporium medicaginis* is reported as approaching *M. meliloti*, a similarity that must have referred to shape since size

[4] For his efforts in trying to locate a specimen the writer is greatly indebted to Gabriele Goidanich, of the Vegetable Pathology Station at Rome, Italy.

is quite different, makes it unlikely that this is a report of *S. sarcinaeforme* attacking *Medicago sativa*.

An echinulate-spored fungus has been collected on alfalfa by several investigators in this country and in each case the fungus corresponds with *Stemphylium botryosum*, particularly as regards the echinulate character of the spores. Krakover states (*9, p. 279*), regarding material he examined from collections belonging to the herbarium of the United States Department of Agriculture,

> Slide mounts of spores on material collected in Philadelphia, Pa., and Arlington Farm, Va. (Turkestan alfalfa) contained spores which in shape and color are the same as the spores of *Macrosporium sarcinaeforme* on clover, but they are smaller in size and decidedly warty.

The writer has examined spore mounts of material furnished by F. R. Jones, which was collected on alfalfa in 1915 at Brookings, S. Dak., Geneva, N. Y., Ithaca, N. Y., and Mercer, Maine. In each case the spores were definitely echinulate. Thus it appears that *Stemphylium botryosum* has been collected on alfalfa several times and is probably more prevalent on that host than on red clover.

Malkoff (*10*) and Gentner (*5*) described a disease of red clover and alfalfa, and in each case they considered *Macrosporium sarcinaeforme* the etiological factor. Gentner obtained the perfect stage of the organism in culture and also collected it from old stems in the field. He identified it as *Pleospora herbarum*. Hülsenberg (*7*) reported a disease on red clover and alfalfa and considered it identical with the one described by Gentner and therefore attributed its cause to *M. sarcinaeforme*. In each of these cases, however, it was reported that the conidia of the causal fungus were echinulate. Since the conidia of *Stemphylium sarcinaeforme* (=*M. sarcinaeforme*) are devoid of warts or spines, it is evident that these investigators were not dealing with that fungus.

In correspondence with Gentner, the writer was advised to write to L. C. Doyer, of Wageningen, Netherlands, for seed infected with *Pleospora herbarum* or *Macrosporium sarcinaeforme*. From diseased seeds of red clover and alfalfa received from Doyer, ascospore cultures of an organism were obtained that appear identical with the organism described by Gentner (*5*). Cultures of the fungus grown on sweetclover stems at a temperature of 10° to 12° C. produced perithecia in about 10 to 14 days and ascospores in about 4 to 5 months. Except for a small difference in size, the measurements of the ascospores (32µ to 46µ by 14µ to 20µ) agree well with those of *Pleospora herbarum*, thus confirming Gentner's (*5*) opinion that the organism is that species. Very few conidia were produced on synthetic media, but abundant conidiophores and conidia were produced on leaf lesions. The conidia and conidiophore characters are very similar to those of *Stemphylium botryosum* described above on red clover and alfalfa, and the conidial stage is therefore considered to be that species. While conidia of the organism obtained from Doyer and conidia from the organisms collected from red clover and alfalfa show small differences in shape, these differences are not considered significant in view of the variation in shape of spores produced by each fungus (fig. 4, *A*, *B*, and *D*). In general the spores of the European culture are slenderer than those of the fungi collected by the writer. This difference is seemingly nullified, however, by the fact that practically as much

difference in spore shape occurs within a culture as between cultures. Gentner (5) observed considerable variation in size and form of spores collected from plants of different origin and pointed out that these characteristics are of little diagnostic value. Furthermore, when single-spore cultures from spores of either the slender type or the short thick type are used to inoculate plants, conidia of both types are produced on the leaf lesions by each type of isolate.

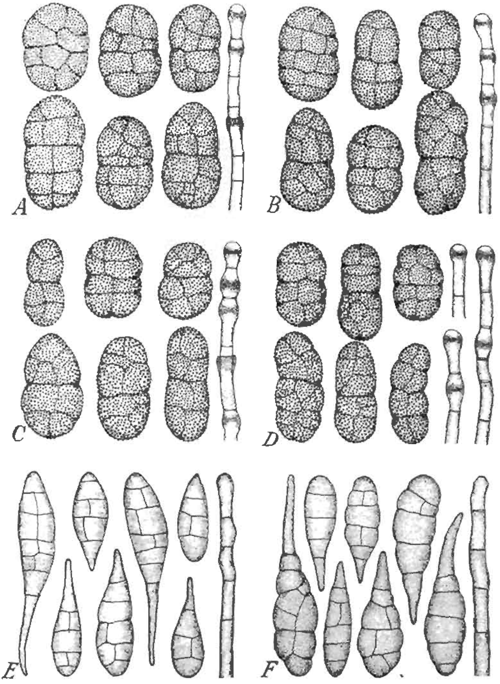

FIGURE 4.—Conidiophores and conidia: *A*, *Stemphylium botryosum* (conidial stage of *Pleospora herbarum*) collected from diseased red clover leaves; *B*, *S. botryosum* collected from diseased alfalfa leaves; *C*, *S. botryosum* from specimen of diseased alfalfa leaves, contributed by L. R. Tehon under the name *Thyrospora sarcinaeforme*; *D*, *S. botryosum* isolated from diseased red clover seeds obtained from L. C. Doyer; *E*, spores of a fungus described by Peck as *Macrosporium meliloti* on *Melilotus alba*; *F*, spores of *Macrosporium commune* from asparagus stems, collected by J. B. Ellis and deposited in the University of Wisconsin herbarium. × about 650.

Macrosporium meliloti Pk. (*14*) is described as occurring on living leaves of *Melilotus alba*. Since inoculations described earlier in this paper showed that *Stemphylium botryosum* is weakly pathogenic on sweetclover, it was thought that this might be the organism described by Peck. A small portion of the material collected by Peck was submitted to the writer by H. D. House. Spores obtained from the specimen are beaked (fig. 4, *E*) and therefore belong to the genus *Alternaria* as defined by Wiltshire (*23*), leaving no doubt that this fungus is different from those considered earlier in this paper. Spores of *Macrosporium meliloti* are very similar to those of *M. commune* Rabh. (fig. 4, *F*) collected by Ellis [5] on asparagus, and the two fungi probably belong in the same species of *Alternaria*.

Another organism that seems to warrant consideration here is *Pleospora lycopersici* É. and É. March. (*11*). This organism is reported (*11*) to be the perfect stage of *Macrosporium sarcinaeforme*, and in the figures that accompany the original description of the fungus the conidia are represented as smooth. This was probably an oversight, however, since Ramsey (*15*) isolated from tomatoes an organism that he considered the same as the one described by Marchal and Marchal, and the conidia are echinulate. Middleton (*12*) has recently shown by inoculation tests with *P. lycopersici* and *M. sarcinaeforme* on tomato and on a number of leguminous plants that the two organisms are distinct. Therefore *P. lycopersici* is not the perfect stage of the smooth, spored organism that occurs on red clover and it is doubtful that the perfect stage of this organism has as yet been found. The organism, described and illustrated by Ramsey, is very similar in both the conidial and perithecial stages to *P. herbarum* collected from red clover and alfalfa. A culture of *P. lycopersici* obtained from Ramsey was used to inoculate red clover and alfalfa by putting a small piece of agar and mycelium on the leaves and placing the plants in a glass-enclosed chamber for 4 or 5 days. The fungus proved slightly pathogenic on red clover but was less virulent than *P. herbarum* isolated from that host. No infection was obtained on alfalfa.

SUMMARY

Stemphylium botryosum Wallr. is reported herein as the cause of a foliage disease of red clover and alfalfa in Wisconsin.

The perfect stage of this fungus was found on dead red clover stems, and cultures from the typical echinulate conidia produced perithecia and ascospores when grown on sweetclover stems or potato-dextrose agar. The ascigerous stage is here identified as *Pleospora herbarum*.

The taxonomic relationship of this fungus to other similar fungi reported on these host plants is discussed.

Evidence is presented which indicates that physiologic races of the fungus exist on red clover and alfalfa.

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THE UTILIZATION BY CALVES OF ENERGY IN RATIONS CONTAINING DIFFERENT PERCENTAGES OF PROTEIN AND IN GLUCOSE SUPPLEMENTS [1]

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INTRODUCTION

A most important problem in the utilization of food energy by animals relates to the effect of combining individual feeds into rations. When rations are thus compounded, the value of the mixture in satisfying the energy requirements of an animal may not be the weighted mean of the values of the individual feeds. This is true, in the first place, because of the associative effects of feeds in digestion. The existence of these effects (but not their causes) has been amply established. In the second place, the supplementing effects of nutrients in metabolism may enhance the utilization of food energy. The realization of this latter fact is a relatively recent accomplishment in the science of nutrition. A historical description of its development has been given elsewhere (*25*).[3]

The effect of a variable intake of protein upon the utilization of food energy during growth was clearly stated by Møllgaard in 1929 (*29*) and demonstrated by the same investigator in earlier experiments on dairy cows. Visco (*34*) in 1930 showed that adult rats on protein-containing diets gained in body weight on energy intakes even smaller than those that, when provided by diets deficient in protein, could not maintain body weight. Hogan (*15, 17*) and Forbes (*7, 10, 11*) and their associates have shown in various publications that, on equal intakes of food, growing rats will make greater gains and, in the Pennsylvania experiments (*7, 10, 11*), store more energy on rations containing moderate concentrations of protein (20 to 25 percent) than on rations containing low and obviously inadequate concentrations. Hamilton (*13*) confirmed these observations and in addition (*14*) proved that, for growing rats, the net availability of the metabolizable energy consumed increased as the percentage of protein (whole egg) in the diet increased up to about 16 percent, remained practically constant throughout the range of protein concentrations within which the diets were equally well-balanced, and then decreased markedly as the dietary protein was increased above 30 percent. These experiments of Hamilton support the general proposition that the better balanced a diet is with reference to a particular combination of animal functions, the smaller will be its energy wastage, represented in the heat increment, and the greater will be its net-energy value, digestive wastages remaining the same.

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[2] The authors wish to acknowledge gratefully the assistance in these experiments of Dr. F. I. Nakamura in the analysis of samples of chamber air.

[3] Italic numbers in parentheses refer to Literature Cited, p. 862.

The purpose of the experiments reported in this paper was to extend to beef calves the study of the relationship between the protein content of rations and the utilization of their energy content that had previously been carried out only with rats and dairy cows in milk. Also, since glucose supplements were used to dilute the protein content of the various basal rations used, an opportunity existed to determine the effect of this sugar on energy metabolism and to compute its net-energy value in the conventional way when incorporated in rations of diverse content of protein.

EXPERIMENTAL PLAN AND METHODS

The plan of the experiment involved the testing of 4 experimental rations, consisting essentially of combinations of corn, cottonseed meal, and timothy hay to provide roughly 6, 10, 15, and 20 percent of crude protein. Each of these rations was fed to each of 4 steer calves at a level to produce small positive energy balances, and immediately after such tests each basal ration was fed with a glucose supplement. The metabolizable energy in each ration (both basal and supplemented) was determined during a period of constant feed lasting from 13 to 28 days, and the heat production was determined in a 3-day period in the respiration chamber. In addition, the fasting heat production was measured for each steer at 5 different times during the experiment. Thus, the entire experiment included 32 collection periods for the determination of metabolizable energy and 52 respiration tests, the combination of which permitted the calculation of net energy.

The subjects of the experiment were four Hereford calves weighing initially from 400 to 500 pounds. They were confined in air-conditioned metabolism stalls, previously described (*16*, *26*), and were fed twice a day, at 9:30 a. m. and 4:30 p. m. They were weighed and watered each morning before feeding and watered again before the second feeding. The heat production was measured in an open-circuit type respiration chamber, also described in an earlier publication (*27*). In the respiration tests on feed, the steers were fed at 8 a. m., immediately after the beginning of the experimental day, and at 4 p. m., water being before them at all times. The tests on feed lasted 3 consecutive days. All fasting experiments were carried out on the fourth and fifth days of fast.

TABLE 1.—*Composition of the four experimental basal rations* [1]

Constituents	Ration containing—			
	6 percent [2] of crude protein	10 percent of crude protein	15 percent of crude protein	20 percent of crude protein
	Percent	*Percent*	*Percent*	*Percent*
Timothy hay	76.4	47.3	39.2	30.0
Ground corn	23.6	47.3	39.2	30.0
Cottonseed meal	0.0	5.4	21.6	40.0

[1] Hereafter called rations 6, 10, 15, and 20.
[2] For steer 1, the percentages were 92.3 and 7.7, respectively, for timothy hay and ground corn.

The composition of the four basal rations is given in table 1. Each basal ration was supplemented with glucose to produce rations that will be referred to as rations 6s, 10s, 15s, and 20s. In the last three of these supplemented rations each steer received daily 1,200 gm. (1,093 gm. of dry matter) of sugar in addition to the same amount of the corresponding basal ration as was consumed in the preceding test, except for ration 10s for steer 2, which included only 1,000 gm. of glucose. Because of its unpalatability, ration 6 was not readily consumed and could not be supplemented with as much glucose as the rations containing larger percentages of protein. Ration 6s for steers 1, 3, and 4, therefore, included only 400 gm. of glucose and for steer 2, 1,000 gm. While on each ration, each steer received daily 28 gm. of sodium chloride, 56 gm. of tricalcium phosphate, 30 gm. of dried yeast, and 10 gm. of a fortified cod-liver oil. The average protein content of the various rations, all supplements included, is given in table 2. The average composition and gross-energy content of the timothy hay, corn, cottonseed meal, and glucose used throughout the experiment are given in table 3.

In order to avoid possible age effects on the utilization of energy, the rations were fed in different order to the various steers, as indicated in table 4, which also gives the average body weights of the steers during both the collection periods and the respiration tests, which in most cases followed the corresponding collection periods. Each experimental feeding period was preceded by a preliminary period on the same amount of feed, varying in length from 5 to 18 days, with three exceptions when the preliminary feeding lasted only 3 days, i. e., for steer 2 on ration 15 and steer 3 on rations 10s and 20s. The dry matter and gross energy cousumed daily in the various experimental periods are shown in table 5.

TABLE 2.—*Average protein content of dry matter of basal and supplemented rations*

Fed —	Rations 6[1] and 6s	Rations 10 and 10s	Rations 15 and 15s	Rations 20 and 20s
	Percent	*Percent*	*Percent*	*Percent*
Unsupplemented	7.53	9.94	15.57	22.41
Supplemented with glucose	7.13	7.69	11.85	16.20

[1] For steer 1, the protein content of the unsupplemented ration was 6.77 percent.

TABLE 3.—*Average percentage composition and gross-energy content of feeds*

Feed	Dry matter	Crude protein	Crude fiber	Gross energy per gram
	Percent	*Percent*	*Percent*	*Calories*
Timothy hay	91.41	5.53	33.46	4.052
Ground corn	88.79	9.21	2.01	3.985
Cottonseed meal	92.90	40.31	10.93	4.557
Glucose	91.09			3.322

TABLE 4.—*Order of feeding experimental rations and average body weights of steers on different rations*

Steer No.	Ration	Order of feeding	Average body weight of steer during—		Ration	Order of feeding	Average body weight of steer during—		Ration	Order of feeding	Average body weight of steer during—		Ration	Order of feeding	Average body weight of steer during—	
			Collection period	Respiration period			Collection period	Respiration period			Collection period	Respiration period			Collection period	Respiration period
			Kilograms	*Kilograms*			*Kilograms*	*Kilograms*			*Kilograms*	*Kilograms*			*Kilograms*	*Kilograms*
1	6	First	211	204	6s	Second	218	219	10	Third	208	211	10s	Fourth	225	230
2	6	Third	228	224	6s	Fourth		232	10	First	206	209	10s	Second	229	224
3	6	Fifth	285	291	6s	Sixth	301	304	10	Third	259	261	10s	Fourth	274	278
4	6	Seventh	316	315	6s	Eighth	327	333	10	Fifth	297	297	10s	Sixth	313	309
1	15	Fifth	228	233	15s	Sixth	254	159	20	Seventh	254	260	20s	Eighth	272	280
2	15	Seventh	266	271	15s	Eighth	290	296	20	Fifth	235	224	20s	Sixth	264	259
3	15	First	216	223	15s	Second	251	251	20	Seventh	297	305	20s	Eighth	323	335
4	15	Third	269	284	15s	Fourth	289	286	20	First	237	239	20s	Second	263	269

TABLE 5.—*The dry matter and gross energy consumed daily in the experimental periods when steers were on different rations*

Steer No.	Ration	Dry matter consumed daily	Gross energy consumed daily	Ration	Dry matter consumed daily	Gross energy consumed daily	Ration	Dry matter consumed daily	Gross energy consumed daily	Ration	Dry matter consumed daily	Gross energy consumed daily
		Kilograms	*Calories*		*Kilograms*	*Calories*		*Kilograms*	*Calories*		*Kilograms*	*Calories*
1	6	2.946	12,835	6s	2.899	12,424	10	2.807	12,399	10s	3.869	16,240
2	6	3.232	14,191	6s			10	2.721	12,108	10s	3.751	15,811
3	6	3.954	17,377	6s	4.304	18,551	10	3.152	13,937	10s	4.237	17,880
4	6	4.097	17,959	6s	4.504	19,383	10	3.617	16,063	10s	4.732	19,833
Average		3.557	15,590		3.922	16,786		3.074	13,627		4.147	17,441
1	15	3.139	14,159	15s	4.245	17,949	20	3.002	13,701	20s	4.119	17,743
2	15	3.331	14,952	15s	4.455	19,020	20	2.868	12,910	20s	3.961	16,932
3	15	2.797	12,681	15s	3.899	16,550	20	3.110	14,188	205	4.228	18,246
4	15	3.186	14,362	15s	4.275	18,292	20	2.736	12,512	20s	3.913	16,840
Average		3.113	14,038		4.218	17,955		2.929	13,328		4.055	17,440

The fasting periods were interposed among the various feeding periods in such a way as to secure information concerning the effects of high and low levels of prefeeding on the heat production during the fourth and fifth days of a following fast. One fasting period preceded all feeding periods for each steer and two fasting periods were run after the regular schedule of feeding, one immediately after the last experimental period, which for all steers was a period in which a sugar-supplemented ration was fed, and the other following an adequate feeding period of at least 2 weeks on the same ration minus its sugar supplement. The other fasting periods were, for all steers, interposed between the second and third and the sixth and seventh feeding periods.

All rations during each period and all collections of urine and feces were analyzed for nitrogen and their heats of combustion (gross energy) determined in an adiabatic Parr oxygen-bomb calorimeter. A few were analyzed also for crude fiber by the official method of the Association of Official Agricultural Chemists.

All calorimetric calculations were made on the basis of daily heat productions corrected to a standard day of 12 hours standing and 12 hours lying down, following the procedure introduced by Armsby.

TABLE 6.—*Fasting heat production of the steers expressed in calories per day per* $W^{.75}_{kg}$

Steer No.	Heat production for fasting period No.—					Average
	1	2	3	4	5	
	Calories	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
1	64.53	56.39	79.05	76.15	64.42	68.11
2	71.39	74.92	78.03	81.24	71.76	75.47
3	72.99	81.87	70.17	71.99	69.19	73.24
4	70.34	78.45	69.59	67.18	65.29	70.17
Average	69.81	72.91	74.21	74.14	67.67	71.75

EXPERIMENTAL RESULTS

FASTING HEAT PRODUCTION

The fasting heat production (table 6) varied from period to period in a disturbing and unexplainable fashion, an experience that Forbes and his associates also recorded (*8*). The values showed no correlation with the average respiratory quotients prevailing during the test nor with the level of feeding immediately preceding the test. The coefficient expressing the correlation between the fasting heat production per day and the daily dry matter consumed in the immediately preceding period, both expressed to the body weight in kilograms raised to the 0.75 power, was +0.188 for the 20 determinations. According to Fisher (*5, p. 176*), for a probability no smaller than 0.1 that the indicated correlation is a result of the operation of fortuitous factors only, the coefficient should be as large as 0.36. It may reasonably be concluded, therefore, either that no correlation exists or that it is quite inappreciable in comparison with other determining factors.

For the purpose of computing the heat increments of the basal rations, the average heat production for each steer per $W^{.75}_{kg.}$ was used, as given in the last column of table 6. The variations in fasting heat production were thus not considered in the calculations simply because

the heat increments obtained by so doing were considerably more variable than those obtained on the assumption of a constant basal metabolism.

The day's heat production dropped from the fourth to the fifth day of fast in 16 of the 20 tests, but on an average this decrease amounted to only 3.44 percent, possibly of the same order of magnitude as the decrease in body weight. The average respiratory quotient for the fourth day of fast was 0.727, and for the fifth day, 0.739.

HEAT INCREMENT OF GLUCOSE AFTER FASTING

Following the second fasting period for each steer, glucose was given as follows, and the heat production determined for the subsequent 12 hours, or, in one case, 24 hours: Steer 1, 100 gm. at 8 a. m., 12 m., and 4 p. m.; steer 2, 500 gm. at 8 a. m. and 2 p. m., though of the latter only 65 gm. was consumed; steer 3, 500 gm. at 8 a. m.; steer 4, 500 gm. at 8 a. m. For steer 3, sugar feeding was repeated after the third fasting period, 600 gm. being given at 8 a. m. The respiration results pertaining to these sugar tests are summarized in table 7.

TABLE 7.—*Effect of glucose feeding on the fasting metabolism of steers*

Steer No.	Heat production during fast [1]		Glucose given	Heat production [2] after sugar feeding	Average respiratory quotients		
					Fasting		After sugar feeding
	Fourth day	Fifth day			Fourth day	Fifth day	
	Calories	*Calories*	*Grams*	*Calories*			
1	3,101	2,916	300	1,544	0.815	0.857	0.912
2	4,187	4,297	565	2,335	.757	.716	.800
3	5,133	5,029	500	2,508	.711	.712	.781
3	4,947	4,845	600	[1] 4,921	.745	.756	.790
4	5,067	5,040	500	2,492	.736	.705	.792

[1] 24-hour period.
[2] 12-hour period only except for second value for steer 3.

The heat production of the steers was practically unaffected by sugar feeding, or inappreciably affected, except in the case of steer 2, although the respiratory quotients indicated sugar oxidation in all cases. The percentage differences between the heat production (expressed per 24 hours) following sugar feeding and the heat production of the fifth day of fast were +5.7 for steer 1, +8.3 for steer 2, −0.26 and +1.56 for steer 3, and −1.12 for steer 4. The last three deviations are well below the expected (average) percentage deviation between successive days on the same feed (2.65, see p. 858), and the first deviation is not improbably beyond the expected range. If the deviation for steer 2 represents a true specific dynamic effect, its magnitude has been greatly exaggerated by the method of calculation, according to which the heat production of the 12-hour subperiod within which the sugar was given and undoubtedly most of the heating effect was exerted was multiplied by 2.

Tests for sugar in the urine following sugar feeding were negative. In the second test on steer 3, the heat production for the first 12 hours

was 2,561 calories and for the second 12 hours, 2,360 calories. The respiratory quotients in successive 6-hour periods following the consumption of 600 gm. of sugar were 0.764, 0.794, 0.858, and 0.744.

Evidently under the conditions of these experiments, glucose may exert no specific dynamic effect, even though it undergoes oxidation. That sugar oxidation was not more pronounced than the rather small rises in the respiratory quotient indicate may be because "it is more important to replenish the carbohydrate stores than to use the incoming carbohydrate for fuel" (*18*). Sugar feeding did not accelerate the paunch fermentation, since subsequent to glucose ingestion the chamber air contained little or no methane.

USE OF THE FASTING HEAT PRODUCTION IN THE COMPUTATION OF HEAT INCREMENTS OF FEED

The purpose of determining the fasting heat production of the experimental steers was to afford a base for the computation of the heat increments due to feeding. This is a commonly used procedure, both among physiologists (such as Rubner and Lusk) interested in the stimulating effect of nutrients on metabolism, and among nutritionists (such as Benedict) interested in determining the losses of food energy in metabolism as extra animal heat. Forbes and his associates (*9*) have used the same procedure in determining the utilization of feed energy for the maintenance of dairy cattle, but since 1928 these investigators have decided that this procedure is grossly in error. They now believe that the fasting heat production of an animal consists of two components, a hypothetical minimum basal level of metabolism and an increment representing the waste heat of the body nutrients catabolized during fast. On this understanding, the difference in heat production between the fasting condition and any level of feeding does not give the true heat increment of the food but gives, rather, this increment minus the waste heat saved by the sparing of body nutrients. Originally proposed as a theory to account for the departure from linearity below the point of energy equilibrium of the curve relating heat production in cattle to the intake of dry matter, this understanding of the nature of the fasting catabolism has assumed the character of an established fact in the minds of its proponents, on the basis of which the work of others has been severely criticized (*6, 11, 22*).

It is the intention of the writers to use the classical method of computing heat increments in this paper in estimating the net energy values of the experimental rations. The arguments that have been advanced in favor of the theory of Forbes (*20*) are unconvincing, mainly because the supporting data cited are open to other interpretations than those selected. In other words, the supporting evidence may be considered as in harmony with the theory, but not as demonstrating its truth. The experimental evidence adduced by Kriss, Forbes, and Miller (*22*) is open to criticism on the basis both of the experimental technic used and of the methods of correcting the heat increments of dietary nutrients for the assumed waste energy of body nutrients spared. And finally it may be said that some experimental evidence may be cited that is difficult, if not impossible, to harmonize with the theory.

Steer No.	Ration	Nitrogen balance	Energy balance	Ration	Nitrogen balance	Energy balance	Ration	Nitrogen balance	Energy balance	Ration	Nitrogen balance	Energy balance
		Grams	*Calories*		*Grams*	*Calories*		*Grams*	*Calories*		*Grams*	*Calories*
1	6	+4.79	−675	6s	(−1.00)	------	10	+13.28	+1,090	10s	+3.60	+2,162
2	6	+8.61	+444	6s			10	+12.15	−173	10s	+8.06	+779
3	6	+4.30	+2,798	6s	+6.92	+2,470	10	+6.85	+492	10s	+14.69	+1,405
4	6	+4.91	+1,052	6s	+3.86	+1,798	10	+9.25	+1,838	10s	+8.88	+2,805
Average		+5.65	+905		+5.39	+2,134		+10.38	+812		+8.81	+1,788
1	15	+19.22	+1,774	15s	+19.18	+1,916	20	+13.78	+1,247	20s	+20.85	+2,262
2	15	+15.62	+1,727	15s	+21.65	+1,923	20	+29.42	+1,231	20s	+29.11	+2,356
3	15	+17.88	+836	15s	+23.08	+1,793	20	+19.55	+1,414	20s	+23.95	+2,625
4	15	+9.36	+1,134	15s	+23.32	+3,175	20	+16.70	+987	20s	+18.96	+2,631
Average		+15.52	+1,368		+21.81	+2,202		+19.86	+1,220		+23.22	+2,469

BALANCES OF NITROGEN AND OF ENERGY

Although the attempt was made to feed the various basal rations at the maintenance level, this proved to be more difficult to attain in the time available for the tests than was anticipated, largely because the steers were still in the period of active growth. In the desire to avoid submaintenance feeding in particular, too much of the rations was fed in all cases but two, and positive energy balances were obtained ranging, on an average, from 812 to 1,368 calories per day, and in individual cases from 492 to 2,798 calories per day (table 8), the next highest individual value being 1,838 calories. The results with the lowest protein levels, rations 6 and 6s, cannot, unfortunately, be considered satisfactory because of the unpalatability of the rations and the consequent irregularity in the consumption of food.

The average increases in energy balance brought about by the sugar supplements were 976, 834, and 1,249 calories per day for rations 10s over 10, 15s over 15, and 20s over 20, respectively. The individual differences were quite variable, especially between rations 15 and 15s.

The nitrogen balances increased with increasing percentages of protein from an average of 5.65 to 19.86 gm. per day. The protein-sparing effect of the sugar supplements was evident only with rations 15s and 20s, their nitrogen balances being quite definitely greater than those of the corresponding basal ration.

TABLE 9.—*Losses of chemical energy, and metabolizable energy of different rations fed to steers*

Ration and steer No.	Energy per kilogram of dry matter					Percentage energy			
	Total	Lost in—			Metabolizable	Lost in—			Metabolizable
		Feces	Urine	Methane		Feces	Urine	Methane	
Ration 6:	*Calories*	*Calories*	*Calories*	*Calories*		*Percent*	*Percent*	*Percent*	*Percent*
1	4,357	2,093	138	341	1,784	48.05	3.17	7.82	40.96
2	4,391	1,951	107	354	1,979	44.43	2.43	8.06	45.08
3	4,395	1,544	130	269	2,451	35.14	2.95	6.13	55.78
4	4,383	1,653	130	337	2,264	37.71	2.96	7.68	51.65
Average [1]	4,390	1,716	122	320	2,231	39.09	2.78	7.29	50.84
Ration 6s:									
1	4,286	2,126	77.3	341	1,742	49.61	1.80	7.95	40.64
2									
3	4,251	1,505	113	283	2,349	35.42	2.66	6.67	55.25
4	4,304	1,680	113	335	2,176	39.03	2.63	7.78	50.56
Average	4,280	1,770	101	320	2,089	41.35	2.36	7.47	48.82
Ration 10:									
1	4,417	1,643	84.4	375	2,314	37.20	1.91	8.50	52.39
2	4,450	1,812	92.6	266	2,279	40.72	2.05	5.98	51.22
3	4,422	1,781	91.1	345	2,205	40.28	2.06	7.80	49.86
4	4,441	1,459	106	272	2,604	32.85	2.38	6.13	58.63
Average	4,432	1,674	93.5	314	2,350	37.76	2.11	7.10	53.03
Ration 10s:									
1	4,171	1,356	76.3	211	2,527	32.52	1.83	5.06	60.59
2	4,215	1,854	57.1	206	2,099	43.98	1.35	4.88	49.79
3	4,220	1,649	68.7	297	2,206	39.07	1.63	7.04	52.26
4	4,191	1,473	73.5	303	2,342	35.14	1.75	7.22	55.89
Average	4,199	1,583	68.9	254	2,294	37.68	1.64	6.05	54.63

[1] Omitting the data for steer 1.

TABLE 9.—*Losses of chemical energy, and metabolizable energy of different rations fed to steers*—Continued

Ration and steer No.	Energy per kilogram of dry matter					Percentage energy			
	Total	Lost in—			Metabolizable	Lost in—			Metabolizable
		Feces	Urine	Methane		Feces	Urine	Methane	
	Calories	*Calories*	*Calories*	*Calories*		*Percent*	*Percent*	*Percent*	*Percent*
Ration 15:									
1	4,511	1,374	119	317	2,700	30.47	2.65	7.03	59.8
2	4,489	1,303	114	318	2,754	29.02	2.54	7.09	61.3
3	4,534	1,539	126	283	2,586	33.95	2.78	6.24	57.0
4	4,508	1,641	106	345	2,416	36.40	2.35	7.65	53.6
Average	4,510	1,464	116	316	2,614	32.46	2.58	7.00	57.9
Ration 15s:									
1	4,228	1,223	88.6	293	2,624	28.93	2.09	6.93	62.0
2	4,269	1,260	78.8	348	2,582	29.52	1.85	8.16	60.4
3	4,247	1,346	62.8	241	2,597	31.70	1.48	5.68	61.1
4	4,279	1,270	68.5	322	2,619	29.67	1.60	7.52	61.2
Average	4,256	1,275	7.47	301	2,606	29.96	1.76	7.07	61.2
Ration 20:									
1	4,564	1,319	139	332	2,774	28.90	3.04	7.28	60.7
2	4,501	1,310	122	313	2,757	29.09	2.71	6.96	61.2
3	4,562	1,297	148	349	2,768	28.44	3.24	7.65	60.6
4	4,573	1,380	137	277	2,779	30.17	3.00	6.06	60.7
Average	4,550	1,326	136	318	2,770	29.15	3.00	6.99	60.8
Ration 20s:									
1	4,308	1,126	83.8	396	2,701	26.15	1.94	9.20	62.7
2	4,275	1,266	52.8	228	2,728	29.61	1.23	5.33	63.8
3	4,316	1,123	74.5	329	2,790	26.01	1.73	7.62	64.6
4	4,304	1,360	75.6	260	2,608	31.60	1.76	6.05	60.5
Average	4,301	1,219	71.7	303	2,707	28.34	1.66	7.05	62.9

METABOLIZABLE ENERGY IN THE RATIONS

The wastages of energy in urine, feces, and methane,[4] and the metabolizable energy remaining for the various rations and steers are summarized in table 9. The following points are noteworthy:

The metabolizability of the gross energy of the basal rations increased progressively from ration 6 to ration 20 as the following average percentages show: 50.84, 53.03, 57.96, and 60.86. This is probably not an effect of the increasing protein percentage but a result of the increasing proportion of concentrates and the decreasing proportion of timothy hay.

Although glucose itself is completely absorbable and highly metabolizable, the metabolizable energy as a percentage of the gross energy in the basal rations suplemented with glucose to the extent of about 30 percent of their gross energy is only slightly greater than that of the basal rations alone. These slight differences are in part the result of smaller proportionate losses of energy in the urine, occasioned by a reduction in the proportion of protein in the rations, and in part the result of variable and inconsiderable decreases in the proportionate loss of energy in the feces. It is noteworthy that neither the absolute amounts of methane produced nor the percentage loss of energy in methane was appreciably different for the rations supple-

[4] The methane was determined in the chamber air by analysis, using the gasometric methane analyzer of Carpenter.

mented with glucose as compared with the basal rations. Obviously the glucose supplements, though highly fermentable themselves, did not stimulate fermentation in the paunch insofar as methane production is a criterion.

TABLE 10.—*Net-energy values of rations*

Steer No.	Net energy value											
	Ration	Per kilogram of dry matter	As percentage of metabolizable energy	Ration	Per kilogram of dry matter	As percentage of metabolizable energy	Ration	Per kilogram of dry matter	As percentage of metabolizable energy	Ration	Per kilogram of dry matter	As percentage of metabolizable energy
		Calories	*Percent*		*Calories*	*Percent*		*Calories*	*Percent*		*Calories*	*Percent*
1	6	[1] 1,019	[1] 57.1	6s	......	......	10	1,732	74.9	10s	1,597	62.8
2	6	1,489	75.2	6s	......	.	10	1,463	64.2	10s	1,373	65.4
3	6	2,014	82.2	6s	1,787	76.1	10	1,664	75.5	10s	1,510	68.4
4	6	1,538	67.9	6s	1,614	74.2	10	1,898	72.9	10s	1,685	71.9
Average	.....	1,680	75.1	....	1,701	75.1	.....	1,689	71.9	...	1,541	67.1
1	15	1,859	68.9	15s	1,486	56.6	20	1,884	67.9	20s	1,682	62.3
2	15	2,032	73.8	15s	1,639	63.5	20	1,954	70.9	20s	1,826	66.9
3	15	1,813	70.1	15s	1,646	63.4	20	2,175	78.6	20s	1,978	70.9
4	15	1,800	74.6	15s	1,884	71.9	20	1,918	69.0	20s	1,865	71.5
Average	.....	1,876	71.8	.....	1,664	63.9	....	1,983	71.6	..	1,838	67.9

[1] Not included in the averages because of irregular consumption of food.

NET ENERGY OF THE RATIONS

The net-energy values of the rations, expressed both in calories per kilogram of dry matter and in percentage of the metabolizable energy, are given in table 10. These values have all been computed by the use of heat increments observed above the fasting heat production, estimated in each feeding period from the average body weight of the steer and the average for the steer of the results of the five basal metabolism periods, expressed in calories per $W_{kg}^{.75}$. In the discussion of table 10, the results for rations 6 and 6s will not be considered because of their incompleteness and of their impaired significance brought about by the irregular consumption of food.

Expressed on the dry-matter basis, the net-energy content of the three basal rations increased, on an average, with the increase in protein content, the values in order being 1,689, 1,876, and 1,983 calories per kilogram. However, the metabolizable energy on the same basis also increased—2,350, 2,614, and 2,770 calories (table 9)—so net availability of the metabolizable energy averaged practically the same for all, i. e., 71.9, 71.8, and 71.6 percent. Although the protein content of ration 10, and to a less extent of ration 15, was definitely inadequate for the maximum retention of nitrogen (see table 8), the insufficiency evidently did not impair the utilization of the metabolizable energy.

The growing beef steer thus differs from the dairy cow in milk and from the growing rat in that the metabolizable energy of rations inadequately balanced with respect to protein is just as well utilized as the metabolizable energy of rations containing adequate amounts of

protein. It is possible that in the improved beef breeds of cattle the process of fat formation is just as efficiently performed, at least within certain limits, as the process of growth with respect to the utilization of food energy. In such a case, food energy consumed above the quota of balanced nutrients in the ration that can be used in the synthesis of organic complexes for the production of new body tissue is diverted to the adipose tissues and there synthesized to fat with an efficiency equal to that realized in the growth process. This would mean that the heat increment of nutrients used in fattening is no greater than that of nutrients used in growth. If these heat increments are determined by the plethora of nutrient material in the intercellular fluids, and, for a given intake of food, if the plethora is dependent on the rate of withdrawal of the end products of digestion from these fluids, the equality of utilization of energy in fattening and in growth implies an equality in rates of synthesis of body fat and of protoplasm.

On the same basis of reasoning, Møllgaard's analogous work on dairy cows in milk (*28, pp. 160–180*) would mean that the rate of synthesis of the organic constituents of milk is greater than the rate of synthesis of body fat and of protoplasm, a proposition that is perfectly obvious.

On the other hand, it is not surprising that such an animal as the albino rat, which does not fatten readily, since it has not been selectively bred with that end in view, should construct protoplasm during growth at a faster rate than it can dispose of excess food by synthesis into fat. Hence, of two comparable rations, the one with a content of protein permitting the greater utilization of food energy in growth would produce the smaller heat increment. This Hamilton (*13*) has shown in his carefully controlled experiments.

The variability of the net-energy values for the individual steers and the 6 experimental rations was not extreme. The root-mean-square deviation of all 24 values from their respective means was 127 calories, equivalent to 7.20 percent of the mean net-energy value, i. e., 1,765 calories. As previously reported (*26*), the writers have computed from 4 recent publications of Forbes and his associates an average percentage difference of 7.1 between pairs of determinations on different steers of the net-energy value of rations for maintenance, with individual differences as high as 13.9 percent. The earlier work of Armsby shows individual differences up to 24 percent. Kleiber (*21*) has calculated a coefficient of variation of 11 percent exhibited by a series of 11 determinations of the net-energy value of starch reported by Kellner and Kühn.

The percentage differences between the heat productions during adjacent days in the calorimeter of the steers in this experiment while on feed averaged 2.65 ± 1.30 for 66 comparisons, as compared with an average of 2.73 ± 1.48 for 75 similar comparisons from the work of Armsby and Fries (*1*). The probable errors appended to the above means define a range within which the probability that a single deviation will fall is 0.5.

AVAILABILITY OF THE ENERGY OF GLUCOSE SUPPLEMENTS

By the usual methods of estimating the metabolizable and net energy of supplemental feeds added to a basal ration, the energy values of the glucose supplements to the various basal diets used in these experi-

ments have been determined, making allowances for differences in the body weights of the steers in comparable periods and for small differences in the consumption of the basal rations. The results of these estimations are summarized in table 11.

Table 11.—*Metabolizable and net-energy values of the glucose supplements in the different rations fed*

Rations from which computed and steer No.	Glucose calories in percentage of basal calories	Energy per kilogram of dry matter		Percentage net availability of metabolizable energy
		Metabolizable	Net	
Rations 10 and 10s:	*Percent*	*Calories*	*Calories*	*Percent*
1	32	3, 131	1, 250	40
2	26	1, 536	1, 094	71
3	28	2, 209	1, 066	48
4	25	1, 471	978	66
Average	28	2, 087	1, 097	56
Rations 15 and 15s:				
1	29	2, 402	410	17
2	25	2, 054	431	21
3	31	2, 624	1, 219	46
4	28	3, 210	1, 895	59
Average	28	2, 572	989	36
Rations 20 and 20s:				
1	29	2, 500	1, 117	45
2	31	2, 654	1, 488	56
3	28	2, 853	1, 416	50
4	31	2, 166	1, 737	80
Average	30	2, 543	1, 439	58

The great variability exhibited by the individual estimates, particularly the low net-energy values for steers 1 and 2 on ration 15s, are to be ascribed in part to the fact that the glucose supplements could not be fed in amounts greater than 1,200 gm. if consistently complete consumption was to result with all basal rations. This amount on the gross-energy basis was equal to only 30 percent of the basal ration. Indirect determinations of heat increments under such conditions cannot possess great accuracy. The variability may also be a result of the extreme irregularity of glucose absorption in the ruminant as revealed by the studies of Von Teichmann and Logischen (*33*) and of Krzywenek and Brüggemann (*23*) on the blood-sugar curve in cattle following glucose feeding. Since sugar is readily fermentable in the paunch, its destruction by the paunch organisms may be expected to vary with conditions not under the control of the investigator. Fraps (*12*) has shown, for example, from a study of digestion coefficients in American experiments published prior to 1924, that the digestibility of crude fiber in ruminants, brought about entirely by the paunch organisms, is two or three times as variable as the digestibility of protein- and nitrogen-free extract.

Because of this variability, average differences in the utilization of the energy of the glucose supplements for the different basal rations cannot be considered significant. The average of all 12 estimates indicates that glucose, with a gross energy value of 3,680 calories per kilogram of dry matter, possesses on the same basis 2,401 calories of

metabolizable energy and 1,175 calories of net energy. An average of 65 percent of its gross energy was metabolizable, while of the metabolizable energy 50 percent was net available.

As far as the writers are aware, calorimetric determinations of the value of glucose in cattle feeding have not been reported in the literature. However, comparison of the above values with those reported for starch is of interest. Armsby and Fries (*2*) have reported 1,636 calories per kilogram as the net-energy value of starch, 81.1 percent of the gross energy being metabolizable, and 49.2 percent of the latter being net available. The results of Kellner and Köhler (*20*), recomputed by Armsby and Fries (*2*), assign a value of 1,803 calories of net energy to the kilogram of starch, with 73.8 percent of the gross energy metabolizable and 58.8 percent of the latter net available. The values found for glucose in the writers' experiments are lower than those reported for starch, the difference being in the metabolizability of the gross energy, which was 65.2 percent for glucose and 81.1 (Armsby) and 73.8 (Kellner) for starch. The net availability of the metabolizable energy was not greatly different for glucose and starch, being 50 percent for the former and 49.2 (Armsby) and 58.8 (Kellner) for the latter.

Kellner (*19*) has reported a net-energy value for sucrose that is 24 percent less than that found for starch and has attributed this deficit to the ready fermentability of sucrose. In the writers' experiments, glucose did not increase the methane production over that of the basal rations (table 9). However, as Woodman and Evans (*36*) report, paunch bacteria are able to ferment glucose much more readily than cellulose, so that glucose would be expected to depress the fermentation of cellulose and other polysaccharides indigestible by body-enzyme action. This would be an associative effect of glucose in digestion that would depress the estimated metabolizable energy of glucose below its true value.

TABLE 12.—*Digestibility of crude fiber in basal rations and in rations supplemented with glucose*

Steer No.	Ration	Digestibility of crude fiber	Ration	Digestibility of crude fiber
		Percent		*Percent*
1	6	51.72	6s	36.15
2	10	36.65	10s	21.35
3	15	35.48	15s	29.70
4	20	35.90	20s	31.76
Average		39.94		29.74

To determine whether such an effect was manifested in this experi-
nent, the digestibility of crude fiber was determined for one of the
teers on each of the basal rations, unsupplemented and supplemented
vith glucose, with the results given in table 12. There was in fact a
listinct depression in the digestibility of the crude fiber, amounting
o more than 25 percent, and this depression would be experienced
iot only by the cellulose contained in the crude fiber but also by that
resent in the nitrogen-free extract (*30*) and probably by the hemi-

celluloses that are also dependent on bacterial action for their digestion.[5]

Hence, it seems fair to conclude that the low metabolizability of the gross energy of glucose by cattle, and presumably other ruminants, is a result both of its own partial destruction by the paunch flora and fauna and of its inhibition of the fermentation of the insoluble polysaccharides of the basal ration that depend upon such fermentation for their digestive utilization. A definite depressing effect of molasses on the digestibility of crude fiber in sheep, quite analogous to the effect of glucose demonstrated in table 12, has been reported by Briggs (*3*)—notwithstanding his conclusions to the contrary—and by Scharrer and Nebelsiek (*31*). No uniformly depressing effect of cane molasses on the digestibility of crude fiber by dairy cows was observed by Williams (*35*). The depressing effect of glucose on the digestion of the insoluble carbohydrates of feeds would not be produced in calves less than 3 months of age because the paunch is not formed until then, according to the X-ray studies of Magee (*24*).

However, the poor utilization of glucose cannot be traced entirely to its effect upon the digestive process in the paunch, since Fingerling and Schoenemann (*4*) and Schoenemann (*32*) have shown that another sugar, sucrose, giving rise to glucose in digestion, has a lower net-energy value than starch, not only for the steer but also for the pig and the dog, in which extensive destruction of carbohydrates by bacterial fermentation does not occur. Furthermore, the ratio of the net-energy value of sucrose to that of starch was not greatly different among these diverse species, being 1:1.37 for the dog, 1:1.28 for the pig, and 1:1.32 for the steer. The nature of this difference between the utilizations of starch and sucrose is unknown and is an interesting subject for future research.

CONCLUSIONS

In experiments upon four steer calves, involving 32 collection periods for the determination of metabolizable energy and 52 tests in the respiration chamber, results were obtained that support the following conclusions:

(1) The utilization of the metabolizable energy in the rations of growing calves is not impaired by inadequate levels of protein within the limits tested. Apparently, the utilization of food energy in fattening is as efficient as that in growth.

(2) Glucose given in moderate amounts to calves that have fasted for 5 days may exert no specific dynamic effect, even though the respiratory quotient indicates its combustion. It does not depress the fasting level of heat production, even though it probably spares body protein. This fact seems incompatible with the theory that the body nutrients catabolized during fasting exert a considerable specific dynamic effect.

(3) The addition of glucose to a basal ration depresses the digestibility of those insoluble carbohydrates (celluloses, hemicelluloses) that depend on the fermentative action of the micro-organisms of the paunch for their digestion. At the same time, the extent of these fermentations, as measured by the production of methane, is not

[5] This marked depression in the digestibility of crude fiber in ruminant animals by the ingestion of glucose has been confirmed by similar (unpublished) experiments on sheep.

increased. This effect is apparently a result of the substitution of glucose for insoluble carbohydrates as the substrate of the bacteria of the paunch.

(4) Because of this associative effect between glucose and the insoluble carbohydrates of the basal ration, estimations of the metabolizability of the gross energy of glucose supplements, averaging 55 percent, are much lower than the true values.

(5) Under the conditions of feeding followed in this experiment, about 50 percent of the metabolizable energy of glucose supplements to a basal ration is wasted in the increment in heat production thus induced. This wastage is comparable to that reported from other laboratories for starch and sucrose.

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TWO TYPES OF FALL PANICUM SMUT[1]

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INTRODUCTION

The common smuts are characterized by black or brown, dusty masses of chlamydospores that break out on the surface of the infected plants. A few cases have been recorded, however, in which the sori of the common smuts have been composed of hyaline chlamydospores and hence were light-colored instead of dark. Campagna,[2] in 1926, reported the finding of a single smutted head of wheat at Ste. Anne de la Pocatiere, Quebec, Canada, which morphologically showed all the symptoms of the ordinary loose smut (*Ustilago tritici* (Pers.) Rostr.), but differed from it by the fact that the spores were hyaline and the spore mass was white instead of black. His attempts to germinate the spores all failed and no artificial inoculations were tried, so that no conclusions could be reached as to the pathogenicity and stability of the organism. He suggested that the white smut was perhaps a case of mutation or sudden variation.

Holton,[3] in 1931, reported a buff type of oat smut in which the chlamydospores were smooth and colorless, in contrast to the dark-brown chlamydospores of the common type of oat smut. This "buff" smut was produced by a cross between two monosporidial lines that originated from interspecific hybrid chlamydospores (*Ustilago avenae* (Pers.) Jens. × *U. levis* (Kell. and Sw.) Magn.). Further studies led Holton [4] to conclude that the buff smut was a result of mutation in *U. levis* and that the change involved only the factor that determines color in the chlamydospores.

Moore and Allison,[5] in 1935, reported the finding of a single smutted head of barley at St. Paul, Minn., infected with what appeared to be an albino strain of *Ustilago hordei* (Pers.) Kell. and Sw. The head was almost white and was intermediate between the loose smut and the covered smut types. The spores were hyaline, smooth, and somewhat smaller than those of normal *U. hordei*. They produced promycelia and sporidia on germination.

Plants of fall panicum (*Panicum dichotomiflorum* Michx.) systemically infected with a smut fungus are abundant at Arlington Experiment Farm, Arlington, Va., each autumn. A healthy and a smutted shoot of this grass, collected in October 1937, are shown in figure 1. The smut fungus agrees well with the description of *Sorosporium*

[1] Received for publication October 11, 1940.

[2] Campagna, E. "NEW WHITE SMUT" OF WHEAT, COLLECTED AT STE. ANNE DE LA POCATIERE. Quebec Soc. Protect. Plants Ann. Rpt. (1925–26) 18: 71–72. 1926.

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[5] Moore, M. B., and Allison, C. C. AN ALBINO STRAIN OF BARLEY SMUT. (Abstract) Phytopathology 25: 27–28. 1935.

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syntherismae (Pk.) Farl. given by Clinton[6] and by Whetzel and Jackson.[7] Although this smut is reported to occur commonly throughout the eastern part of the United States on various species of *Cenchrus*

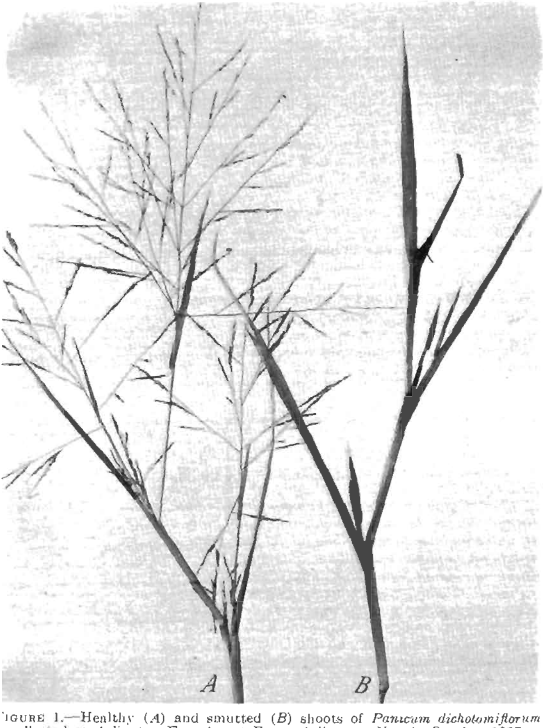


FIGURE 1.—Healthy (*A*) and smutted (*B*) shoots of *Panicum dichotomiflorum* collected at Arlington Experiment Farm, Arlington, Va., in October 1937.

nd *Panicum* and is known also from Mexico, South America, and Bermuda, it appeared that little study had been made of it. Consequently a supply of the dark chlamydospore material was collected

[6] CLINTON, GEORGE PERKINS. ORDER USTILAGINALES. North Amer. Flora 7: 1-82. 1906.
[7] WHETZEL, H. H., and JACKSON, H. S. THE RUSTS AND SMUTS OF BERMUDA. Brit. Mycol. Soc. Trans. : 1-32. 1928.

and an inoculation experiment was planned to determine the effect of incubation-period temperature on infection.

In the course of the experiment a buff type of smut was observed by the writers. The present paper records the results of an investigation of the pathogenicity of both types and of the relationship between them.

EFFECT OF INCUBATION-PERIOD TEMPERATURE ON PATHOGENICITY OF SOROSPORIUM SYNTHERISMAE

Seed of fall panicum, collected at Arlington farm in October 1937, was dusted with smut chlamydospores (*Sorosporium syntherismae*) on February 23, 1938, and was sown thickly in 4-inch pots of soil. Similarly prepared pots were sown with noninoculated seed as checks, and the pots were placed in pairs in chambers maintained at 5°, 10°, 15°, and 20° C. After 26 days under these conditions, the pots were all assembled on a greenhouse bench. Two weeks later the grass seedlings were transplanted individually to thumb pots and were permitted to grow in these under greenhouse conditions until June 22, 1938, when counts were made to determine the percentages of infection. The results of these counts (table 1) show that after the inoculated seed had been incubated for 26 days at 5°, 10°, 15°, and 20° the percentages of plants developing smut were 70.9, 72.5, 53.3, and 22.2 percent, respectively. The grass seed used in this experiment was not treated previous to inoculation, but only 4 smutted plants developed out of a total of 571 check plants grown from the noninoculated seed (table 1).

TABLE 1.—*Effect of incubation-period temperature on the pathogenicity of Sorosporium syntherismae on Panicum dichotomiflorum at Arlington Experiment Farm, Arlington, Va., in 1938*

Incubation period temperature (° C.)	Plants from inoculated seed			Plants from noninoculated seed		
	Total	Smutted		Total	Smutted	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
5	189	134	70.9	260	1	0.4
10	193	140	72.5	194	2	1.0
15	60	[1] 32	53.3	78	0	.0
20	9	2	22.2	39	1	2.6

[1] 1 smutted plant in this lot had buff sori with hyaline, smooth chlamydospores; all other smutted plants in the experiment had black sori with brown, echinulate chlamydospores.

COMPARISON OF DARK AND BUFF TYPES OF SOROSPORIUM SYNTHERISMAE

MORPHOLOGIC DIFFERENCES

While the counts were being made in the above-described experiment, it was observed that one smutted plant in the inoculated lot incubated at 15° C. had buff sori. The striking difference in the appearance of these two types of smut sori is shown in figure 2, *A* and *B*. The shredding of the host tissue and the balls of hyaline chlamydospores characteristic of the buff-type smut are shown in the enlarged view of a buff sorus (fig. 3).

Microscopic examination of the chlamydospores composing the two types of sori revealed not only a difference in color but also a difference in the spore wall. The spores of the buff sori are hyaline and smooth-walled, while those of the common black sori are brown and echinulate or minutely verrucose. These differences are shown in the photomicrographs (fig. 4, *A* and *B*). The hyaline spores are almost spherical in shape, while the brown spores are subspherical or somewhat

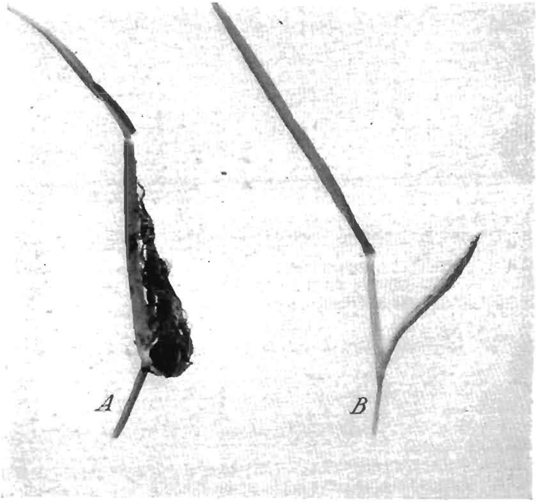
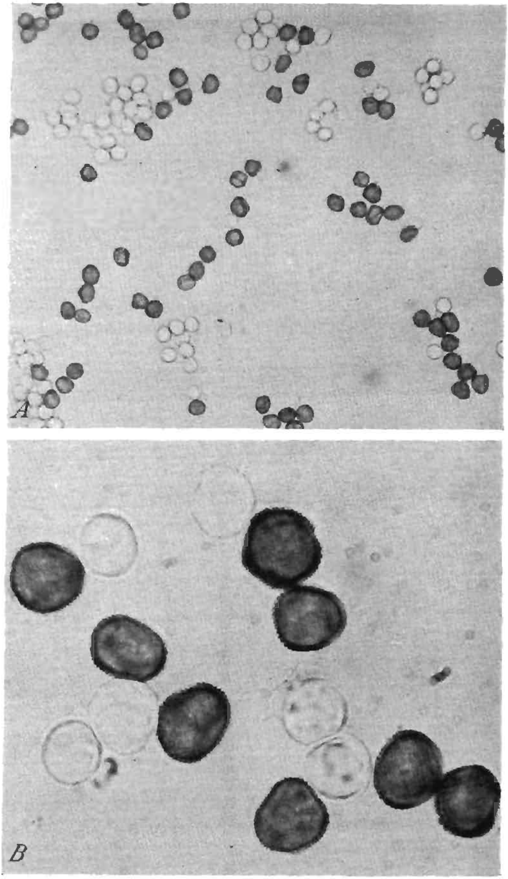


FIGURE 2.—Two types of smut that developed when seed of fall panicum was inoculated with chlamydospores of *Sorosporium syntherismae* collected in the field at Arlington Experiment Farm, Arlington, Va., in 1938: *A*, Common dark-type sorus; *B*, buff-type sorus. Both × 1.

polyhedral. Although the hyaline chlamydospores appear appreciably smaller than the brown when examined under the microscope, measurements failed to reveal any great differences in size. One hundred hyaline chlamydospores had an average diameter of 11.9μ, while a hundred brown chlamydospores averaged 12.7μ by 11.9μ. In a second set of measurements, 25 hyaline spores averaged 11.8μ by 11.5μ, whereas 25 brown spores averaged 12.4μ by 11.5μ. These averages all fall within the 9μ to 13μ spore size recorded by Clinton[8] for *Sorosporium syntherismae*.

[8] See footnote 6.

FIGURE 3.—Buff-type sorus of *Sorosporium syntherismae* showing shredding of host tissue and balls of hyaline chlamydospores. ×10.



GURE 4.—Photomicrographs showing the contrast in color and cell wall between individual chlamydospores of the buff type and of the common dark type of *Sorosporium syntherismae*: *A*, ×350; *B*, ×1,500.

CULTURAL DIFFERENCES

Single chlamydospore cultures of the two types of smut were established and distinct cultural differences were found to exist. The culture of the black smut was of the sporidial type with a pronounced tendency to sector (fig. 5, *A*) whereas the culture of the buff smut was mycelial and quite stable (*B*). To determine the effect of tempera-

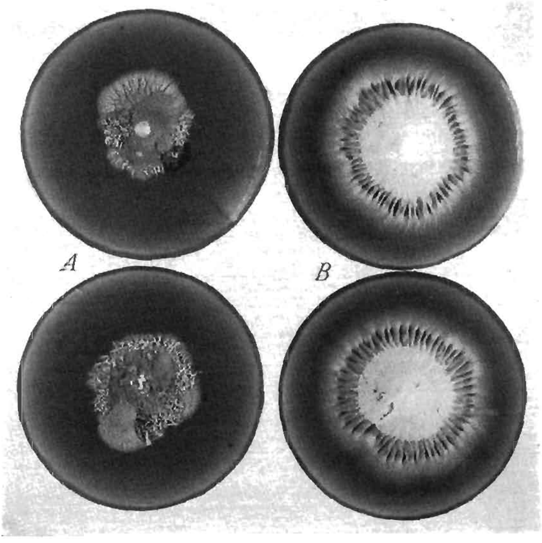


FIGURE 5.—Cultural differences of brown (*A*) and hyaline (*B*) single chlamydospore lines of *Sorosporium syntherismae* grown on potato-dextrose agar for 30 days at 20° C. in 1939.

ture on the growth of a hyaline and a brown single chlamydospore line of *Sorosporium syntherismae*, the fungus was grown on potato-dextrose agar in Erlenmeyer flasks for 30 days. During this period the flasks were located in electric incubators at a temperature range of 5° to 35° C. in 5° intervals.

The average growth in millimeters made by the two types of cultures at each of the seven temperatures employed is shown graphically in figure 6. The similarity in shape of the two curves (fig. 6) shows that the cardinal points for the growth of these two types of cultures are approximately the same. Both grow slowly at 5° C. and have a

distinct optimum near 20° and a maximum somewhere between 30° and 35°. The greater radial growth of the buff smut culture was apparently due to its mycelial nature, while the sporidial type culture of the black smut tended to pile up rather than to spread radially. It is interesting to note that the optimum temperature for the growth of this fungus in culture (20°) is considerably higher than the optimum temperature for infection, which was shown in the inoculation experiment discussed above to be from 5° to 10°.

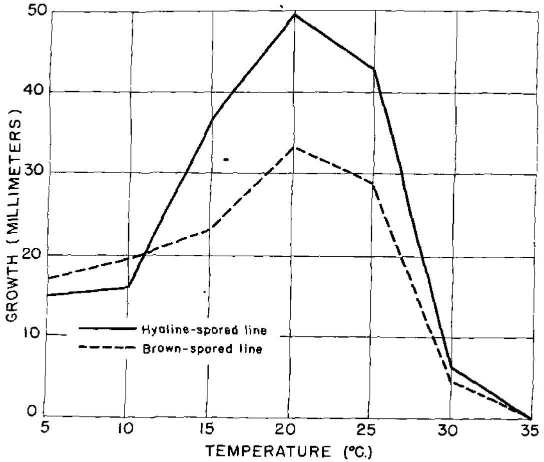


FIGURE 6.—Effect of temperature on the growth of a hyaline and a brown single chlamydospore line of *Sorosporium syntherismae* grown on potato-dextrose agar for 30 days in 1939.

EFFECT OF METHOD OF INOCULATION AND INCUBATION-PERIOD TEMPERATURE ON PATHOGENICITY

On March 6, 1939, an experiment was started to determine the effect of method of inoculation and incubation-period temperature on the pathogenicity of the buff type and of the common dark type of *Sorosporium syntherismae*. On this date seed of fall panicum was given parallel inoculations with the dark-type smut and the buff type. The following methods were used with each type: The seed was (1) dusted with dry chlamydospores, (2) wet with a suspension of chlamydospores, and (3) placed under partial vacuum in a suspension of chlamydospores. The inoculated seed was then planted at once in

4-inch pots of soil, as in the 1938 inoculation experiment, and the pots were placed in chambers maintained at 10° and 20° C. Similarly prepared pots were sown with noninoculated seed as checks and were placed in the same chambers as the treated pots. In the pots held at 20° the seedlings emerged between March 14 and 21, and these pots were transferred to a greenhouse bench during this period. In the pots held at 10° the seedlings had emerged by April 1, and on that date these pots were placed on the greenhouse bench with the others. The incubation period in this experiment thus varied from 8 to 26 days in length as compared with the uniform period of 26 days employed in the 1938 inoculation experiment (p. —).

Three weeks after the final lot of pots was moved from the incubation chambers to the greenhouse, the grass seedlings were transplanted individually to thumb pots and were permitted to grow in these under greenhouse conditions until June 9 to July 17, 1939, during which period counts were made to determine percentages of infection. The results of these counts are summarized in table 2. The data presented in table 2 show that in general a higher percentage of smutted plants developed after an incubation period of 10° C. than after an incubation period of 20°, thus confirming the results of the 1938 experiment. These data show further that dusting the grass seed with dry chlamydospores resulted in more smutted plants than did either of the moist methods of inoculation. It would appear from these data, also, that the common dark type of smut is somewhat more pathogenic than is the buff type, since with the latter type of inoculum percentages of infection were lower in all but one instance. The noninoculated check plants in this experiment all remained free from smut.

The 68 smutted plants which resulted from inoculation of seed with chlamydospores of the buff-type smut all produced only buff sori (fig. 7, *A* and *B*). This is considered further evidence of the stability of the buff-type smut. The 240 smutted plants that resulted from inoculation of seed with chlamydospores of the common dark-type smut all produced only black sori in this experiment. A typical black sorus that resulted from inoculation with dark chlamydospores is illustrated in figure 7, *C*.

Table 2.—*Effect of method of inoculation and incubation-period temperature on the pathogenicity of the buff type and the common dark type of Sorosporium syntherismae on Panicum dichotomiflorum at Arlington Experiment Farm, Arlington, Va., in 1939*

Method of inoculating seed	Incubation-period temperature	Plants from seed inoculated with— Buff-type spores: Total	Buff-type spores: Smutted		Common dark-type spores: Total	Common dark-type spores: Smutted	
	° C.	Number	Number	Percent	Number	Number	Percent
Dusted with chlamydospores	10	77	29	37.7	196	167	85.2
	20	59	14	23.7	96	28	29.2
Wet with suspension of chlamydospores	10	165	8	4.9	121	3	2.5
	20	100	2	2.0	69	4	5.8
Partial vacuum, using suspension of chlamydospores	10	67	10	14.9	74	36	48.6
	20	47	5	10.6	99	2	2.0
Noninoculated seed	10	60	0	.0	167	0	.0
	20	53	0	.0	115	0	.0

'IGURE 7.—Buff-type sori (*A* and *B*) and common dark-type sorus (*C*) that developed when seed of fall panicum was inoculated with hyaline, smooth chlamydospores and brown, echinulate chlamydospores, respectively, in 1939. ×1.

SUMMARY AND CONCLUSIONS

An experiment was made at Arlington Experiment Farm, Arlington, 'a., in 1938, to determine the effect of incubation-period temperature n the pathogenicity of *Sorosporium syntherismae* (Pk.) Farl. on *'anicum dichotomiflorum* Michx. After seed dusted with chlamydo-)ores had been incubated for 26 days at temperatures of 5°, 10°, 5°, and 20° C., the percentages of smutted plants developing were).9, 72.5, 53.3, and 22.2 percent, respectively.

One smutted plant in this experiment was observed to have buff)ri composed of hyaline, smooth-walled chlamydospores, while the :her 307 smutted plants all had the common black sori composed of :own, echinulate-walled chlamydospores.

Single chlamydospore cultures of the two types of smut were tablished. The cultures of the buff-type smut were mycelial and able, while those of the common black-type smut were of the ›oridial type with a pronounced tendency to sector. The optimum mperature for the growth of each type on potato-dextrose agar was ›proximately 20° C., and the maximum for each was between)° and 35°.

Inoculations made in 1939 with chlamydospores of the buff-type smut resulted in 68 smutted plants, all of which produced only buff sori. Parallel inoculations with chlamydospores of the dark-type smut resulted in 240 smutted plants, all of which produced only black sori.

It is concluded that the buff smut is a result of mutation in *Sorosporium syntherismae* and that the change may involve several genetic factors, since the mutant differs from the common dark smut in spore color, marking of spore wall, spore shape and size to a minor extent, and in degree of pathogenicity.

COMPARISON OF THE WOOL AND SKINS OF FULL-FED AND MAINTENANCE-FED LAMBS [1]

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INTRODUCTION

It has been known since the early work of Buchtala (*6*) [2] that keratin-containing substances, such as wool, hair, nails, horns, and skin, are particularly rich in the sulfur-containing amino acid cystine. As emphasized by Harris and Smith (*11*), the cystine content is, to a high degree, a measure of the stability and quality of wool. Rimington (*16*), in fact, proved that cystine sulfur accounts for practically all the sulfur in wool. This finding has been confirmed by Sullivan and Hess (*18*), who found that the cystine content of the wool tested accounted for 97.1 percent of the total sulfur. However, differences have been observed by Rimington (*16*) in the cystine content of the wool from different breeds of sheep, and also by Ramaiyya (*15*) and Marston (*13*) in the sulfur content and the rate of growth of wool from the same breed of sheep but on different diets. According to Fraser (*10*), the effect of nutrition on wool growth and cystine content is still a moot question.

An opportunity to test the effect of diet on the cystine content of both wool and skins was afforded by a feeding experiment on lambs conducted at the United States Department of Agriculture Beltsville Research Center, Beltsville, Md. The results obtained are reported in this paper.

HANDLING OF THE ANIMALS AND SKINS

The animals used comprised nine pairs of twin Rambouillet wether lambs born at the United States Range Livestock Experiment Station, Miles City, Mont., in the spring of 1933. The lambs were selected for uniformity in age, weight, type, form, grade, and fleece. On the day of weaning (September 15, 1933), the lambs were shipped to Beltsville, Md., where they arrived on September 29. Experimental feeding was begun on October 13. The animals were kept indoors with only slight exposure to direct sunlight but were allowed the run of an outdoor lot for a 2-hour period daily except in stormy weather. They were fed individually, in separate pens for 112 days, a diet consisting of shelled corn, 7 parts by weight; and cottonseed meal, 1 part; corn silage; and clover hay. Water and rock salt were given ad libitum. One animal of each of the nine pairs of twin lambs was full-fed, and the other nine animals received the same diet but in quantities that permitted only a

[1] Received for publication October 3, 1940. A preliminary report on this work in relation to wool was presented before the American Society of Biological Chemists in 1935 (*22*). The authors are indebted to J. I. Hardy of the Bureau of Animal Industry, United States Department of Agriculture, for the sectioning and examination of the fibers according to a technique developed by him, and to F. W. Frey of the Bureau of Chemistry and Soils, now the Bureau of Agricultural Chemistry and Engineering, for the samples of hides that he had prepared for use in a study of the histological structure of the same hides (*7*).

[2] Italic numbers in parentheses refer to Literature Cited, p. 885.

slight increase in weight. A summary of the experimental feeding is shown in table 1.

TABLE 1.—*Average daily feed consumed, body weights, and weights of fleeces and skins of the full-fed and maintenance-fed lambs* [1]

Item	Full-fed lambs	Maintenance-fed lambs
Daily feed consumed.	*Pounds*	*Pounds*
Shelled corn and cottonseed meal (mixture of 7:1)	1.20	0.33
Corn silage	.60	.59
Clover hay	1.01	.56
Salt	.012	.023
Water	3.38	1.54
Initial body weight	47.90	48.00
Final weight after 112 days of feeding	88.70	49.90
Weight of scoured fleece	2.62	1.88
Weight of whole skins	7.88	3.72

[1] Data obtained by V. L. Simmons, of the Bureau of Animal Industry, in connection with the management of the animals.

At the end of the experiment (February 2, 1934) the wool was collected separately from each lamb. If soiled, the samples were "skirted" before being analyzed, that is, soiled edges were sheared. The skins were weighed immediately after removal from the carcass. The left sides were cured, in four piles, with fine new meat salt for 25 days, at the end of which time they contained 14 to 16 percent of salt. All were in good condition except that the top piece of each pile had become dry. Six of the sides, three from the maintenance-fed lambs and three from the twin full-fed lambs, were preserved without further treatment. The skins from the full-fed lambs were thicker and heavier than those from the maintenance-fed lambs; the average weights are given in table 1.

The bend area, or about half of the total area of these skins, was shaved with a razor to remove the wool stubbles. Part of this shaved area was used for the estimation of cystine and sulfur after about 2 years of storage at the Bureau of Chemistry and Soils, now the Bureau of Agricultural Chemistry and Engineering.

EXPERIMENTS INVOLVING CYSTINE AND SULFUR CONTENTS

ANALYTICAL PROCEDURE WITH WOOL

Analyses of the cystine content of the wool and of the cystine and sulfur contents of the skins were made at Georgetown University. For convenience of analytical work the wool from only six paired sets of lambs was taken. Analyses of the various samples (from May 17 to July 24, 1934) were made by the same analyst without any knowledge of the significance of the particular samples.

Before analysis the individual samples of wool were freed from lipoidal matter by means of warm benzol according to the procedure recommended by Rimington (*16*). For the analysis, air-dried samples of the benzol-free water-washed wool were taken of the whole fibers, and also of the whole fibers cut into thirds. The subdivision was made on the assumption that whatever difference might occur as a result of differences in the diets would be manifested in the inner third of the fibers.

The whole fibers were hydrolyzed with 20-percent hydrochloric acid for 6 hours in an oil bath maintained at 125° C. The individual thirds were similarly hydrolyzed. Cystine was determined in the respective hydrolyzates by the Sullivan (*17*), the Okuda (*14*), the Folin-Marenzi (*9*), and the total-sulfur methods, the last mentioned one being determined by the Benedict (*2*) method.

The cystine methods differ in their degree of specificity. The Sullivan method is one of high specificity for free cystine and is negative with cystine tied or altered in any way. This method, in fact, requires that the three groups of cysteine (formed from cystine by reduction) be unsubstituted and in the order as in natural cysteine, $HS—CH_2CH(NH_2)COOH$. It is negative with cystine amine, which contains no carboxyl group, and with homocystine, through which might pass methionine considered to be converted to cystine in the animal body.

The Okuda iodometric method gives a positive result with various disulfides, with cystine congeners in which the S-S linkage is still reducible (such as cystine amine and homocystine), and with nonsulfur compounds of high reducing capacity. In hydrolyzates of purified proteins in general, however, Sullivan and Hess (*18*) have found that the Okuda method closely parallels the Sullivan method.

The Folin-Marenzi method for cystine is less specific than the Okuda or the Sullivan methods and in a number of cases has been found to give too high cystine values (*16, 17, 18*).

ANALYTICAL PROCEDURE WITH SKINS

The salted skins of three pairs of the lambs, after being cut into small pieces (diced), were extracted in a Soxhlet apparatus with ether freed from alcohol and peroxides. The fat-free skins were then extracted with water until they were free from sodium chloride. They were then filtered by suction and dried in a desiccator over calcium chloride.

The skins were hydrolyzed with 20-percent hydrochloric acid for 6 hours in an oil bath maintained at 125° C. The hydrolyzates of the skins contained a considerable quantity of humin, in contrast to the wool hydrolyzates, which contained practically no humin. Accordingly, the skins were hydrolyzed in a reducing atmosphere, that is, with hydrochloric acid containing titanous chloride—a type of hydrolysis that was found by Sullivan (*17*) and by Sullivan and Hess (*19, 21*) to prevent humin formation in a number of proteins. The procedure employed in the present case was as follows: Two grams of each finely cut skin was hydrolyzed for 4 hours with 4 cc. of 20-percent hydrochloric acid and 2 cc. of 20-percent titanous chloride, in an oil bath maintained at 125° C. No humin was observed. The solutions were carefully neutralized with 5 N sodium hydroxide added dropwise with stirring, until no more blue precipitate (titanous hydroxide) occurred. After filtration, part of the filtrate was used for cysteine determination and part for the determination of the total sulfur of the hydrolyzate by the Benedict (*2*) method.

RESULTS WITH WOOL

Table 2 gives the data, obtained by the Sullivan method, for the cystine content of the whole fiber and the various parts of the fiber for each of the animals. Similar analyses were made by the Okuda,

Folin-Marenzi, and total-sulfur procedures. Since all these findings corroborated, in general, those found by the Sullivan method, only the average results are given in table 3.

TABLE 2.—*Cystine content of wool from six pairs of twin lambs, one twin maintenance-fed and the other full-fed (determinations by the Sullivan method)*

MAINTENANCE-FED LAMBS

Lamb No.	Cystine content of— Whole fiber	Outer third of fiber	Middle third of fiber	Inner third of fiber	Treatment of wool
	Percent	*Percent*	*Percent*	*Percent*	
1344	11.70	11.55	11.77	11.21	Skirted.
1348	11.57	11.96	10.11	8.86	Do.
1430	12.39	12.14	11.10	11.40	Do.
2030	12.05	12.03	11.92	11.13	Unskirted.
2260	11.40	11.49	11.43	11.28	Skirted.
2326	11.49	11.08	11.62	11.47	Do.
Average	11.77	11.71	11.32	10.89	

FULL-FED LAMBS

Lamb No.	Whole fiber	Outer third of fiber	Middle third of fiber	Inner third of fiber	Treatment of wool
1343	11.78	11.02	12.34	11.87	Unskirted.
1349	10.87	10.39	10.90	11.47	Skirted.
1431	12.32	11.66	11.99	12.55	Do.
2029	12.15	11.91	12.15	12.60	Unskirted.
2259	10.88	11.17	11.03	10.42	Skirted.
2327	12.31	10.93	11.64	13.29	Do.
Average	11.72	11.18	11.68	12.03	

TABLE 3.—*Average cystine and sulfur contents of wool from maintenance-fed and full-fed lambs, as determined by various methods*

Method of determining cystine content	Method of feeding lambs	Cystine content of— Whole fiber	Outer third of fiber	Middle third of fiber	Inner third of fiber
		Percent	*Percent*	*Percent*	*Percent*
Sullivan	Maintenance-fed	11.75	11.70	11.32	10.89
	Full-fed	11.72	11.18	11.68	12.03
Okuda	Maintenance-fed	11.94	11.84	11.51	11.13
	Full-fed	11.97	11.39	11.86	12.50
Folin-Marenzi	Maintenance-fed	13.01	12.54	13.32	12.57
	Full-fed	12.37	12.48	13.09	14.51
Total sulfur [1]	Maintenance-fed	12.45	12.24	11.90	11.58
	Full-fed	12.04	11.71	12.01	12.90

[1] The sulfur content of the wool is expressed as cystine by multiplying the value for total sulfur by 3.75, the factor to convert sulfur to cystine.

In the hydrolyzates obtained by the Sullivan method the samples of whole fibers, both the long fibers from the lambs on the full diet and the shorter fibers from those on the maintenance diet, had, on the average, practically the same cystine content. Secondly, in the lambs on the maintenance diet the cystine content tended to decrease from the outer third to the inner third of the fibers, whereas in the full-fed lambs it tended to increase.

The Okuda method gave, in these hydrolyzates, results of the same order of magnitude as the Sullivan method. In the Okuda method,

also, the average cystine contents of the whole fiber of both sets of lambs were practically identical. On the other hand, considering the average cystine content of the inner third of the wool from the lambs on the full ration as 100, that of the inner third from the lambs on the maintenance diet was 89.

In a 6-hour hydrolyzate, the Folin-Marenzi method for cystine frequently gives values higher than that for total sulfur. This result was obtained in the hydrolyzates of the wool from a number of the lambs studied. Despite the impossible high values found in the hydrolyzates of some of these samples of wool, the findings by the Folin-Marenzi method were in general agreement with those of the Okuda and the Sullivan methods. Also, considering the cystine content of the inner third of the wool of the full-fed lambs as 100, that of the inner third of the wool of the maintenance-fed lambs was 87.

Similarly, determining the total sulfur on aliquots of these hydrolyzates and computing this sulfur as cystine, as given in table 3, showed, on the average, practically the same percentages for the whole fibers of the lambs fed the maintenance and full diets. On the other hand, the cystine content of the inner third of the wool from the full-fed lambs and from those on the maintenance diet was in the ratio of 100:90.

Every method of testing showed that the inner third of the wool fibers from the lambs on the full diet was about 10 percent higher in cystine than the inner third of the wool fibers from the lambs on the maintenance diet.

In cross sections of the wool fibers, the diameters of the wool of the maintenance-fed lambs were less than those of the full-fed lambs, but there was no difference in the structure nor evidence of increased medullary space when the fibers were examined either in cross section or longitudinally by polarized light. It appears, therefore, that the lowered cystine content of the inner third of the wool from the maintenance-fed lambs is the result of the change in the protein of the cortex and not an increase of the medullary space and its associated constituents.

RESULTS WITH SKINS

As it was found that the inner third of the wool from the full-fed lambs gave higher cystine and sulfur value than the inner third of the wool from the maintenance-fed lambs, a similar comparison of the relative cystine and sulfur contents was made of the skins of three maintenance-fed and three full-fed lambs. As stated previously, the ordinary hydrolysis with 20-percent hydrochloric acid gave a considerable quantity of humin, which called for decolorization and gave results for cystine slightly lower than when humin formation was prevented. The comparative results, however, were of the same order of magnitude with the hydrochloric acid hydrolysis as with the hydrolysis involving hydrochloric acid containing titanous chloride. Since the latter is considered the better procedure, in the present case only the findings by this method are given in table 4. As determined by iodometric titration before and after reduction with zinc and hydrochloric acid (Okuda method), no cystine was present in the hydrolyzate. With cysteine as the standard the cysteine in the hydrolyzate was determined and computed as cystine.

TABLE 4.—*Cystine and sulfur contents of the skins of maintenance-fed and full-fed lambs*

Lamb No.	Cystine content by—		Total sulfur content in—	
	Sullivan method	Okuda method	Skin	Hydrolyzate
MAINTENANCE-FED LAMBS				
	Percent	*Percent*	*Percent*	*Percent*
1430	0.66	0.67	0.52	0.50
1454	.45	.46	.64	.61
2260	.79	.81	.54	.46
Average	.63	.65	.57	.52
FULL-FED LAMBS				
1431	0.98	1.00	0.75	0.71
1455	.70	.71	.82	.76
2259	.95	1.01	.80	.77
Average	.88	.91	.79	.75

There were distinct differences between animals, but the skins of the lambs on the full diet individually and collectively gave much higher cystine and sulfur values than the skins from the lambs on the maintenance diet. The average ratio of the cystine content of the skins from the lambs on the latter diet to that of the lambs on the former was 100:140 with the Sullivan colorimetric method and the Okuda iodometric method. The ratio of the total sulfur of the skins for the two groups of lambs was, on the average, 100:139. The relative agreement of the two methods shows that no disulfide other than cystine was present.

In the skins the cystine contains only part of the total sulfur. Although there is no information on the methionine content of lambskins, the stratum corneum of human skin has been shown by Wilkerson and Tulane (*29*) to contain at least as much methionine as cystine. The difference between the two groups of experimental animals is brought out more markedly in the skins than in the wool from the lambs on the respective diets.

There are, in biochemical literature, various analyses of the cystine content of human skin. Among these are the following: Abderhalden and Zorn (*1*) for psoriasis scales, 1.85 percent; Wilkerson (*28*) for human epidermal scales, 2.31 percent; Eckstein (*8*) and Block (*4*) 3.82 and 3.40 percent, respectively, for outer layers of human skins. For human skin, Wilson and Lewis (*30*) report a cystine content of 1.8 to 2.3 percent with no details as to whether the skins were entire skins or mainly outer layers.

Since the lambskins utilized in the present work contain closely packed wool roots and included inner and outer skin and connective tissue, it seems a questionable procedure to compare the results with the work of others. However, mention is made of one analysis, by Sullivan and Hess,[3] of a sample of the entire skin obtained from a man accidentally killed. The skin sample freed from fat by acetone and ether extraction, and analyzed by the methods used for the lambskins gave a cystine value of approximately 0.9 percent, a result comparable with that of the skins from the lambs on the full diet.

[3] Unpublished data.

EXPERIMENTS INVOLVING HISTIDINE, LYSINE, AND ARGININE CONTENTS

Since there was such a marked difference in the contents of cystine and total sulfur in the skins from the twin lambs on the two levels of feeding, it was considered desirable to determine the contents of the basic amino acids histidine, lysine, and arginine. Although several investigators—Wilkerson (*28*), Eckstein (*8*), and Block (*4*)—have reported on these three amino acids in human skin, no work on the basic amino acids of lambskins has come to the present authors' attention. Vickery and Block (*24*) found that wool contained 0.66 percent of histidine, 2.3 percent of lysine, and 7.6 percent of arginine, and later (*25*) reported the molecular ratio of these three amino acids for wool to be 1:4:12.

ANALYTICAL PROCEDURE

Analyses for histidine, lysine, and arginine were made of the whole fibers of the wool from one pair of twin lambs: Lamb No. 1430, on the maintenance diet; and No. 1431, on the full diet. The methods used were those of Vickery and Leavenworth (*26, 27*), including the modifications introduced by Vickery and Block (*25*) and Block and Vickery (*5*).

Similar analyses were made of the skins of two pairs of the twin lambs. The skins, which, as previously stated, had been preserved in rock salt, were handled as follows: All of them were defatted with anhydrous peroxide-free ether. To determine the effects of the salt on the analytical results, a portion of each skin from one of the two pairs (Nos. 1430 and 1431) was then washed with water to remove the salt, dried in a desiccator over calcium chloride, and hydrolyzed with 8 N sulfuric acid. The remainder of these two skins and the second pair were similarly hydrolyzed without being washed.

The histidine and arginine were isolated as the flavianate and the lysine as the picrate. The percentage of each amino acid was calculated from the weight of the dried flavianate and picrate.

RESULTS

In the wool analyses, the percentages of histidine, lysine, and arginine were 0.71, 2.64, and 9.47, respectively, for the maintenance-fed lamb and 0.71, 2.66, and 9.57 for the full-fed lamb. The molecular ratio of these amino acids is 1:4:12, as found by Vickery and Block. The histidine and lysine contents of the wool were similar to those given by Vickery and Block (*24*). The arginine content was higher than that obtained by these investigators but is in better agreement with that (10.4 percent) reported recently by Vickery (*23*) and obtained by the use of the diflavianate method.

Table 5 gives the results of the analyses of the skin after correction for the moisture and ash in the skin samples.

The table shows that the washed and unwashed samples gave values of the same order of magnitude for histidine, lysine, and arginine. In other words, the presence of 14 to 16 percent of salt in the samples made relatively little difference in the analytical results. Although there is some variation in the percentages of histidine, lysine, and arginine in the skins from the full-fed and maintenance-fed lambs, the molecular ratio is of the same order of magnitude for both pairs of

lambs, being 1:4.4:16 for the unwashed skins of the full-fed lambs and 1:5.2:16 for those of the maintenance-fed lambs.

TABLE 5.—*Histidine, lysine, and arginine contents of the skins of maintenance-fed and full-fed lambs*

Lamb No.	Treatment of skin	Histidine	Lysine	Arginine
MAINTENANCE-FED LAMBS				
		Percent	*Percent*	*Percent*
1430	Washed	0.71	3.36	11.92
	Unwashed	.66	3.12	12.45
2260	Unwashed	.93	3.95	17.40
FULL-FED LAMBS				
1431	Washed	0.73	3.49	11.96
	Unwashed	.74	3.42	13.49
2259	Unwashed	.82	3.36	14.97

Hess (*12*) has shown that the molecular ratio of histidine:lysine:arginine in normal fingernails is of the same order of magnitude (1:6:13) as in arthritic fingernails, whereas, on the average, the cystine content of arthritic fingernails was markedly lower than that of normal fingernails, as reported by Sullivan and Hess (*20*). Much the same result is obtained in lambskins. The cystine and sulfur contents of the skins of the lambs on the maintenance diet were markedly lower than those of the lambs on the full diet. On the other hand, the histidine, lysine, and arginine values are of the same order of magnitude in both sets of skins. Likewise, though the individual skins may differ in the content of the amino acids, the molecular ratios of the basic amino acids histidine, lysine, and arginine are relatively constant. The findings for the skins tend to support the conclusion of Block (*3*) that tissue proteins are built upon or around an "anlage" of relatively fixed proportions of histidine, lysine, and arginine and agree with the ratio found by Wilkerson (*28*) for the stratum corneum of human skin.

SUMMARY

From October 1933 to February 1934, at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., nine Rambouillet wether lambs, each being one of a pair of twins, were full-fed. The others of the nine pairs of twins received the same diet but in quantities that permitted only a slight gain in weight.

The wool collected separately from six pairs of the lambs was analyzed for cystine, at Georgetown University, Washington, D. C., by the Sullivan, Okuda, Folin-Marenzi, and total-sulfur methods. The sulfur content of all the fibers was likewise determined.

All the methods employed agreed in showing that the wool of the lambs on the maintenance diet contained approximately 10 percent less cystine in the body third of the fibers than did the wool of the lambs receiving the full diet. This reduction was in the protein of the cortex, since examination of the fiber failed to show evidence of increased medullary spaces in the wool fibers of the maintenance-fed lambs.

The cystine and sulfur contents of the skins of three pairs of the lambs were likewise determined. The skins of the lambs on the full-

fed diet contained markedly more cystine and sulfur than those of the lambs on the maintenance diet. The effect of the maintenance diet showed more markedly in the skins than in the wool from the lambs on the two diets.

The data indicate that low levels of feeding influence not only the quantity of wool but also the quality, as measured by the cystine content.

As a result of the marked difference in the cystine and total sulfur contents of the skins from the lambs on the two levels of feeding, determination of the contents of the basic amino acids histidine, lysine, and arginine were made with the skins and wool of several pairs of the lambs. As judged by the findings from two pairs of lambs only a slight variation was found in the contents of these amino acids in the lambskins. The molecular ratio of histidine : lysine : arginine was approximately 1 : 5 : 16. As judged by the analysis of the wool of one pair of lambs the basic amino acids of the wool were of the same order of magnitude for the two levels of feeding. The molecular ratio of histidine : lysine : arginine was 1 : 4 : 12.

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DIGESTIBILITY OF NUTRIENTS IN FOUR VARIETIES OF SWEETCLOVER HAY[1]

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INTRODUCTION

A widespread interest in sweetclover (*Melilotus alba* Desr.) as a pasture and a hay crop with the resultant testing of improved strains and varieties has led to a study of the digestibility of the organic nutrients in four biennial varieties of this legume. Digestion experiments of 10 days' duration, each preceded by a 10-day preliminary period, were carried out with yearling ewe lambs fed sweetclover hay produced in 1937. The first- and second-year crops of Alpha 1, Grundy County, Arctic, and Common White varieties were grown simultaneously during 1936 and 1937 and their chemical composition determined. These studies were designed to measure the chemical and digestibility differences in the four varieties of sweetclover.

DESCRIPTION OF SWEETCLOVER VARIETIES

The following varities were selected for investigation: Alpha 1, a fine-stemmed, low-growing, low-branching, leafy variety sometimes used as an orchard cover crop; Grundy County, a leafy, coarse-stemmed, dwarfed type; Arctic, a tall-growing, high-branching variety with a low leaf percentage; and Common White, a tall-growing, high-branching, coarse strain with a low leaf percentage. Both the first- and second-year crops of each variety were harvested during 1936 and 1937 in order to take account of any differences in climate and soil that might have occurred during the two seasons. A height of 36 inches was taken as the standard at the time of cutting for the first-year stands, and the early bud stage for second-year stands. A 2-inch stubble was left each year.

REVIEW OF LITERATURE

Garber and his coworkers (*2*)[2] show that when second-year sweetclover is cut for hay in the prebud stage it makes a more vigorous recovery than when cut later. They point out that to make the best hays, sweetclover should not be permitted to show flower buds before it is harvested. It is important that the second-year crop be cut so high that a new growth will develop. This is in agreement with the recommendations of Lloyd (*4*).

Willard's experiments (*8*) show that sweetclover hay contains 50 to 60 percent of leaves in the fall of the first year and only 25 to 35 percent during the second year. Hawk[3] reports the leaf yield of the first-year crop to be 47 percent for the Alpha 1 variety, 61 percent for Grundy County, and 47 percent for Common Tall White. Second-

[1] Received for publication June 20, 1940. Published as Scientific Paper No. 463, College of Agriculture and Experiment Station, State College of Washington.

[2] Italic numbers in parentheses refer to Literature Cited, p. 891.

[3] Hawk, V. B. THE EFFECT OF CLIPPING ON THE YIELD AND NITROGEN CONTENT OF SWEETCLOVER. 90 pp. 1938. [Master's thesis. Copy on file Wash. State Col. Libr.]

year crops were found to yield 42 percent for Alpha 1, and 31 percent for Common Tall White.

McMichael[4] has shown that Common White sweetclover should not be cut for hay after the late-bud stage, because in the more mature stages the proportion of fiber to protein increases rapidly. Neidig and Snyder (*5*) studied sweetclover clippings and observed a high dry matter and low protein content in the more mature stages.

At the Ohio Experiment Station (*6*) sweetclover contained an average of 8.13 percent of ash, 17.31 percent of protein, 1.18 percent of calcium, and 0.28 percent of phosphorus on a water-free basis. It ranked well with other legumes in its content of calcium and phosphorus.

Lindsey and his coworkers (*3*) fed green sweetclover to young sheep. The samples of forage averaged 84.5 percent of water, and the dry matter contained 7.08 percent of ash, 19.4 percent of protein, 30.29 percent of fiber, 40.1 percent of nitrogen-free extract, and 3.13 percent of fat. When this clover was fed in the early blossom stage, the sheep digested 69.05 percent of the dry matter, 76.93 percent of the protein, 69.57 percent of the fiber, 69.03 percent of the nitrogen-free extract, and 50.10 percent of the fat. A slightly later stage showed a digestibility of 69.64 percent dry matter, 79.21 percent protein, 49.95 percent fiber, 68.48 percent nitrogen-free extract, and 52.28 percent fat. Results of tests with the later stage showed a marked decrease in digestibility of crude fiber. The experiment showed a high utilization of the sweetclover nutrients in a stage just previous to blooming.

CHEMICAL COMPOSITION OF FORAGE SAMPLES

The composition of the water-free samples is shown in table 1. The moisture in the air-dry samples is also indicated. First and second-year Grundy County samples were higher in protein than the other varieties. In all but one instance the second-year samples contained more crude fiber. The calcium and phosphorus figures are very similar to those previously reported by the author (*7*) for alfalfa hay.

TABLE 1.—*Composition of water-free samples of sweetclover hay of four varieties harvested in 1936 and 1937*

Crop and variety	Year's growth	Moisture in air-dry samples	Chemical composition of dry matter						
			Ash	Crude protein (N× 6.25)	Crude fiber	N-free extract	Ether extract	Calcium	Phosphorus
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1936 crop:									
Alpha 1	First	6.47	8.65	13.82	34.09	41.28	2.18	1.26	0.31
	Second	7.92	8.93	15.73	30.85	42.36	2.13	2.37	.32
Grundy County	First	6.73	10.09	17.05	26.72	43.02	3.12	1.80	.35
	Second	6.93	8.94	16.83	33.53	39.03	1.67	1.59	.32
Arctic	First	6.48	8.97	14.83	31.14	42.36	2.70	1.65	.32
	Second	7.21	8.68	14.48	38.40	37.01	1.43	1.56	.30
Common White	First	6.76	9.57	15.69	29.93	42.85	1.96	1.68	.32
	Second	7.34	8.87	15.59	31.69	40.66	3.19	1.87	.31
1937 crop:									
Alpha 1	First	16.81	9.17	15.00	32.10	41.00	2.73		
	Second	17.51	9.40	14.28	33.69	40.46	2.17		
Grundy County	First	19.57	9.31	15.57	29.65	42.77	2.70		
	Second	19.73	9.61	17.78	29.75	40.90	1.96		
Arctic	First	16.57	9.76	14.56	30.82	42.26	2.60		
	Second	17.15	8.91	14.16	38.14	37.14	1.65		
Common White	First	18.29	9.48	14.81	32.76	40.36	2.59		
	Second	23.46	8.69	14.66	33.96	40.87	1.82		

[4] McMICHAEL, S. C. THE AGRONOMIC VALUE OF VARIETIES OF BIENNIAL WHITE SWEETCLOVER. 66 pp. 1932. [Master's thesis. Copy on file Wash. State Col. Libr.]

TABLE 2.—*The apparent digestibility of 4 varieties of sweetclover hay when fed to lambs*

Variety	Year's growth	Lamb No.	Live weight of lambs: Initial	Live weight of lambs: Final	Daily dry-matter intake	Apparent digestibility of—: Dry matter	Protein	Crude fiber	N-free extract	Ether extract	Additional data for 1937 crop computed to a 10 percent moisture basis: Digestibility of crude protein	Total digestibility of nutrients
			Kilograms	*Kilograms*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Alpha 1	First	1	36.7	34.9	255.4	43	65	40	55	31		
		2	45.4	44.4	499.1	57	74	41	67	43		
		3	49.4	49.4	711.3	56	73	40	66	40		
		Average				52.0	70.7	40.3	62.7	38.0	8.80	46.13
	Second	1	34.9	35.1	490.2	55	65	47	63	31		
		2	45.1	44.4	494.9	55	67	46	63	27		
		3	48.8	49.2	705.3	54	67	45	62	20		
		Average				54.7	66.3	46.0	62.7	26.0	9.38	47.18
Grundy County White	First	4	51.3	50.3	667.0	58	72	47	67	42		
		5	49.7	50.8	649.2	57	70	43	67	45		
		6	54.9	52.6	679.6	57	72	42	67	46		
		Average				57.3	71.3	44.0	67.0	44.3	10.94	50.25
	Second	4	49.9	49.9	665.7	62	74	51	70	29		
		5	54.9	53.5	678.3	58	73	45	66	29		
		6	50.4	50.6	647.9	56	70	40	65	30		
		Average				58.7	72.3	45.3	67.0	29.3	10.95	49.30
Arctic	First	4	49.4	49.9	686.3	60	74	45	69	44		
		5	53.1	52.6	700.8	57	73	42	67	38		
		6	51.0	51.0	675.8	57	73	42	68	40		
		Average				58.0	73.3	43.0	68.0	40.7	9.78	50.01
	Second	4	47.8	49.7	683.5	56	66	52	61	21		
		5	52.8	52.8	695.9	53	67	45	59	28		
		6	49.9	51.5	671.1	53	65	47	57	28		
		Average				54.0	66.0	48.0	59.0	25.7	8.61	45.59
Common White	First	1	39.2	37.2	465.7	53	71	37	61	44		
		5	47.6	45.4	488.5	55	73	40	63	45		
		3	49.0	47.8	699.8	57	73	45	67	39		
		Average				55.0	72.3	40.7	63.7	42.7	10.21	47.44
	Second	1	37.2	36.1	425.3	50	62	41	59	7		
		2	46.3	44.7	457.6	48	65	35	58	20		
		3	49.9	48.5	655.5	49	64	38	58	11		
		Average				49.0	63.7	38.0	58.3	12.7	8.93	41.94

DIGESTION EXPERIMENTS

Records of sheep weights and feed intake are summarized in table 2. The same group of lambs was fed both the first- and second-year forage samples of any particular variety in the digestion experiments in order to equalize individual differences. The dry-matter intake during each trial for each lamb in any series was approximately the same (with the exception of lamb 1, trial 1) so that any differences in digestibility that might be attributed to unequal feed intake were reduced to a minimum. The records show quite a uniform relationship of water to dry-matter intake. The protein in the feces ranged from

4.07 to 6.71 percent, the dry matter from 38.40 to 64.10 percent, and the ash from 4.25 to 7.96 percent.

The coefficients of apparent digestibility obtained from 24 individual digestion experiments with lambs are contained in table 2. Averages computed for each trial were used in computing the digestible nutrients of the forages studied.

The results with Alpha 1 sweetclover show a higher digestibility of protein and fat in the first-year hay. The fiber and dry matter were digested slightly better in the second-year hay, no differences being indicated for the nitrogen-free extract.

With the exception of fat, which was digested better in the first-year crop, the nutrients of Grundy County sweetclover were as well digested in the first-year as in the second-year growth.

Arctic sweetclover showed a higher digestibility of dry matter, protein, nitrogen-free extract, and fat in the first-year growth than in that of the second year. The reverse relation was true for crude fiber.

Common White sweetclover of the first-year growth had higher coefficients of apparent digestibility for dry matter, protein, nitrogen-free extract, and fat, than those obtained by feeding the second-year crop.

Grundy County sweetclover appeared to be the only variety studied in which the nutrients of the first-year growth were not digested better than those of the second-year growth.

The percentages of digestible crude protein and total digestible nutrients in the eight samples of hay fed during the digestion experiments are contained in table 2. In order to facilitate comparisons of varieties on a more practical basis, the composition of the forage samples was adjusted to a 10-percent moisture content.

In 1934 the author (7) reported average results for three cuttings of northern-grown, common alfalfa, containing 10 percent of moisture. The digestible protein totaled 9.77 percent and the total digestible nutrients 48.43 percent as determined with sheep. A comparison of the eight samples of sweetclover hay with the nutrients in alfalfa (*Medicago sativa* L.) valued as 100 percent is shown in table 3.

TABLE 3.—*Average digestibility of protein and total digestible nutrients in 3 cuttings of northern-grown alfalfa hay as compared with averages for 8 samples of sweetclover hay of 4 varieties.*

[Values for alfalfa = 100]

Hay	Year's growth	Protein	Total digestible nutrients
Alfalfa (northern-grown, common)		100.0	100.0
Sweetclover variety:			
Alpha 1	First	90.1	95.3
	Second	96.0	97.4
Grundy County	First	112.0	103.8
	Second	112.1	101.8
Arctic	First	100.1	103.3
	Second	88.1	94.1
Common White	First	104.5	98.0
	Second	91.4	86.6

The table shows second-year Common White sweetclover to be the lowest in total digestible nutrients of the eight forage samples.

SUMMARY AND CONCLUSIONS

First- and second-year crops of four biennial varieties of sweetclover were studied in digestion experiments with lambs.

The nutrients of Alpha 1 and of Grundy County White were as well digested in the first- as in the second-year hays. Second-year Arctic had a lower digestibility of dry-matter, protein, nitrogen-free extract, and fat than did the first-year sample. First-year Common White was superior to the second-year crop because the protein and especially the crude fat were better digested. The digestible protein of the eight forages reduced to a 10-percent moisture basis ranged from 8.6 to 10.95 percent, and the total digestible nutrients from 41.94 to 50.25 percent.

With respect to digestible protein the hays ranked in the following order: Second-year Grundy County, first-year Grundy County, first-year Common White, first-year Arctic, second-year Alpha 1, second-year Common White, first-year Alpha 1, and second-year Arctic.

The ratings in respect to total digestible nutrients were as follows: First-year Grundy County, first-year Arctic, second-year Grundy County, first-year Common White, second-year Alpha 1, first-year Alpha 1, second-year Arctic, and second-year Common White. In each case first-year crops occupy three of the first four places. The sweetclover hays contained from 88.1 to 112.1 percent as much digestible crude protein and 86.6 to 103.8 percent as much total digestible nutrients as a good grade of alfalfa hay.

First- and second-year Grundy County, first-year Arctic, and first-year Common White were the ranking varieties in regard to feed value when both protein and total digestible nutrients were considered.

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